# Hydroxamic Acids − An Intriguing Family of Enzyme Inhibitors and Biomedical Ligands

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This review on hydroxamic acids deals with their efficacy as inhibitors of enzymes including cyclooxygenases, their synthesis, the complexity and structural diversity of their metal complexes, and their ability to act as effective nitric oxide donors.



## 1. Introduction

Hydroxamic acids, RCONR′OH, have been known since 1869 with the discovery of oxalohydroxamic acid by Lossen.[1] Despite this, research on these compounds was lacking until the 1980's, after which an enormous amount of information has accumulated with respect to their biomedical applications, their synthesis and the synthesis and structures of their metal complexes. As a result, these weak acids, whether naturally occurring or synthetic, constitute one of the most important families of organic bioligands.

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One of the first physiological roles of hydroxamic acids was associated with their use as siderophores, a class of low molecular-weight iron-sequestering agents. In the early stages of life, iron, an essential element required by proteins for electron transport, oxygen transport and other life sustaining processes, was in much demand but in poor supply, mainly due to its insolubility under physiological conditions. To circumvent this, organisms, mainly microbial and bacterial, developed a method of effectively assimilating iron in soluble form by synthesising and utilising siderophores.[2,3] These compounds possess either hydroxamate or catecholate groups that are capable of coordinating to iron(III) to give water-soluble, very stable, high-spin octahedral complexes. The desferrioxamine B (a trishydroxamate siderophore, Figure 1) complex of iron(III), for example, has a formation constant log $\beta \approx 30.4$.[4] Complexes

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**MICROREVIEWS:** This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

of iron(II) with hydroxamate ligands are much less stable than those of iron(III), e.g. the desferrioxamine-iron(II) complex has a log $\beta \approx 10$.[5] Even under anaerobic conditions, above pH 4, in the iron(II)-desferrioxamine B system, oxidation of iron(II) to iron(III) by hydroxamate occurs to give the iron(III)-desferrioxamine B complex and desferrioxamine B monoamide.[5] The iron siderophore complex, once inside the cell, undergoes reduction, releasing iron and making it accessible to metabolic demands within the cell.[6] A remarkable feature of siderophores, which is crucial to their function, is their ability to selectively bind iron(III) over other metal ions, which may be "poisonous". Siderophores also have high affinities for other tripositive metal ions such as aluminium(III), but their complexes are less stable than those of iron(III).[7]

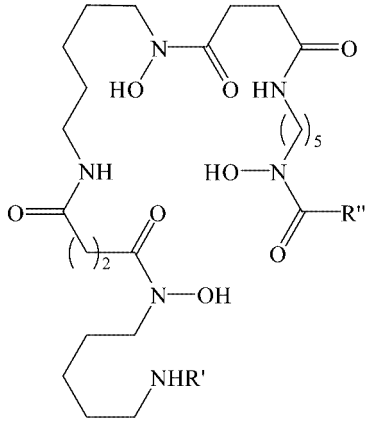



Figure 1. Hydroxamate siderophore, Desferrioxamine B

Siderophores and their analogues have vast therapeutic potential, yet few practical applications of siderophores have been realised. One example is the use of the trishydroxamate siderophore desferrioxamine B (Desferal) to deal with iron-overload in transfusion-dependent patients such as those suffering from thalassaemia.

Thalassaemia, an inherited disease affecting 100,000 babies annually, is characterized by an abnormality in one or more of the globin genes resulting in the inability to synthesise haemoglobin properly, and the patients becoming anaemic.[8] Thalassaemic patients have regular blood transfusions to supplement their haemoglobin levels but this can result in a toxic buildup of iron in the blood, and its accumulation in the heart, liver, endocrine glands and other vital organs eventually resulting in death. Desferrioxamine B, which is administered by prolonged subcutaneous infusion 3 to 7 times per week or by daily intramuscular injections due to its instability in stomach acid, forms a water-soluble iron(III) complex at physiological pH, which is readily excreted in bile and urine. Whilst treatment of thalassaemic patients with regular blood transfusions prolongs their life expectancy by about 20 years, treatment of the resulting iron overload with desferrioxamine B dramatically increases their life expectancy as shown in Figure 2.[9]

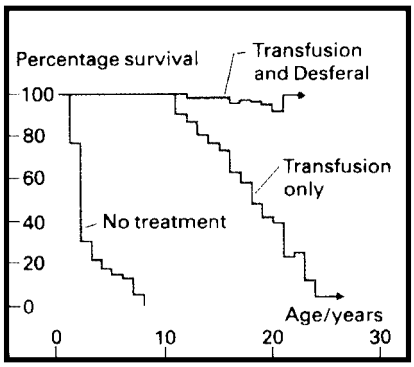



Figure 2. Patient survival rate for treatment of thallasaemia by transfusion only and by both transfusion and administration of desferrioxamine B; taken from ref.[9], reproduced by permission of The Royal Society of Chemistry

The major disadvantage of desferrioxamine B, which has over the past 30 years been the most widely used iron chelator in haematology, is that it is orally inactive.[10] Analogues of desferrioxamine B and a family of compounds known as *N*-hydroxypyrid-2-ones, which are considered to be aromatic hydroxamates are stable in acid and orally active, and are currently being considered as viable alternatives to desferrioxamine B.[11] A related compound deferiprone (not a hydroxamate but a hydroxypyrid-4-one) has been developed as an orally active iron chelator[12,13] but its efficacy has been challenged, and this has resulted in well-publicised acrimony and indeed lawsuits. Analogues of deferiprone are currently under investigation.[11]

Another more recent and potentially powerful application of hydroxamate-containing siderophores is the development of bioconjugates, which are synthesised by covalently linking siderophores with antimicrobial agents. Because microbes have a unique ability to distinguish and employ only certain siderophores, such conjugates could be utilised as carriers to selectively deliver antimicrobial prodrugs to their site of action. The scientific methodology and subsequent syntheses of a number of siderophores, including aerobactin, arthrobactin, schizokinen, a mycobactin, foroxymithine, all of the components of pseudobactin and several analogues, has recently been reported by Miller et al.[14] This group is currently devoting its efforts towards the syntheses and investigation of siderophore antimicrobial agent conjugates in a programme designed to develop iron transport-mediated selective drug delivery systems such as those shown in Figure 3.

This alternative approach to drug delivery may lead to the development of a novel class of selective antimicrobial agents based on active transport of an essential nutrient. The synthesis of such bioconjugates may in addition revitalise old drugs that relied on passive diffusion, and to which resistance has developed. Interestingly, preliminary studies of some of the synthetic siderophore analogues developed by Miller et al. indicate that they have considerable potential as nontoxic, organ selective magnetic resonance imaging (MRI) contrast agents.

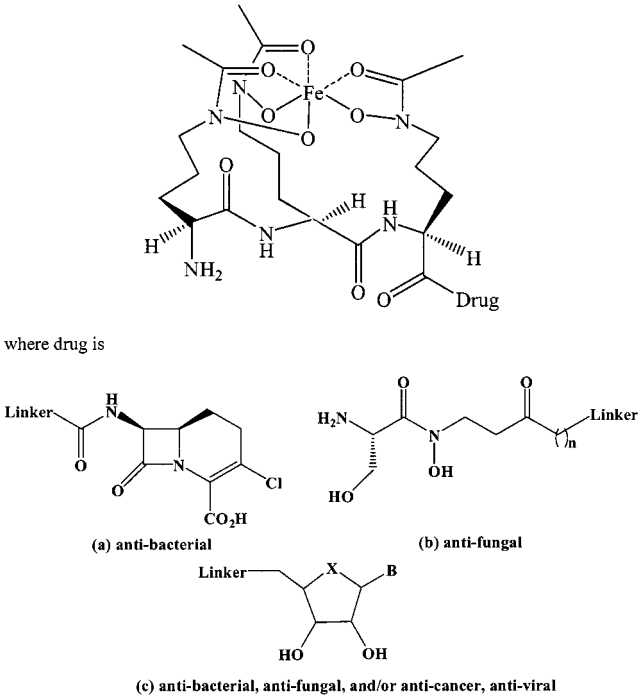



Figure 3. Representative siderophore antimicrobial/anticancer/antiviral agent conjugates as potential iron transport-mediated selective drug delivery systems

The biomedical applications of hydroxamic acids are no longer solely associated with the uptake or removal of iron from the body. In recent years, there has been increasing interest in their roles as potent and selective inhibitors of a range of enzymes including matrix metalloproteases,[15−19] peroxidases,[20−22] hydrolases,[23,24] ureases,[25−28] lipoxygenases,[15] cyclooxygenases[29,30] and peptide deformilases.[31] The basic medicinal chemistry and pharmacology of hydroxamic acid derivatives that act as enzyme inhibitors has recently been the subject of two reviews by Williamson et al.[15] and Lou et al.[32] Hydroxamic acids also represent a wide spectrum of bioactive compounds that have hypotensive,[33] anti-cancer,[16,34−37] anti-malarial,[38−42] anti-tuberculosis and antifungal properties,[43] and have been identified as key functional groups of potential chemotherapeutics targeting cardiovascular diseases, HIV and Alzheimer's disease.[44,45]

Our interest in hydroxamic acids lies in their versatility as ligands, their ability to act as effective nitric oxide donors, and in their potential as specific inhibitors of a class of enzymes known as cyclooxygenases.

## 2. Syntheses and Structures

Hydroxamic acids are generally considered to be derivatives of hydroxylamine and carboxylic acids, although their metal chelating ability is significantly stronger than the corresponding carboxylic acids. Typically, reaction between hydroxylamine or *O*/*N*-protected hydroxylamines (generated in situ by the addition of base to the hydrochlorides) and an activated acyl group (such as an ester, acid halide, amide) results in the formation of the corresponding hydroxamic acid, Equation (1).

$$RCOX + NH_2OH \rightarrow RCONHOH + HX \quad (1)$$

Recently, a convenient two-step procedure for the parallel synthesis of low molecular-weight hydroxamic acids from carboxylic acids and hydroxylamine with the use of polymer-supported 1-hydroxybenzotriazole has been reported (Figure 4).[46]

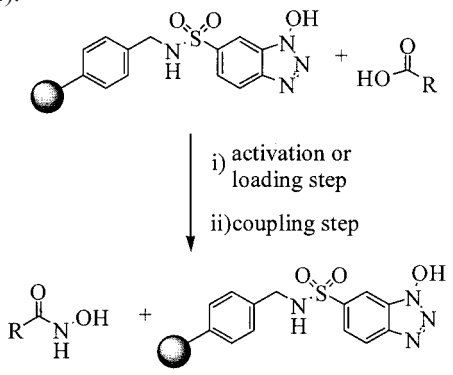



Figure 4. Parallel synthesis of low molecular-weight hydroxamic acids from carboxylic acids and hydroxylamine; reagents and conditions: i. PS-HOBt (1 equiv.), carboxylic acid (1.5 equiv.), DIC (4.5 equiv.), DMAP (0.6 equiv.), DCM/DMF (1:1), 3 h; ii: hydroxylamine (0.6−0.8 equiv.), THF, 5 h

Variations of these methods have been reported and are summarised in a recent paper that describes a simple one-pot method for the syntheses of hydroxamic acids in high yields.[47] The formation of hydroxamic acids and bioactive hydroxamate-containing compounds in both solution and solid phase has also recently been the subject of a comprehensive review by Lou et al.[48]

Two possible hydroxamic acid tautomers exist (Figure 5); the keto tautomer, which is predominant under acidic conditions, and the enol form, which is more stable in alkaline media.[49] Furthermore, NMR spectroscopic studies have shown that each tautomer can exist as (*E*) and (*Z*) isomers (Figure 5), further extending their structural diversity.[50] The conformational preferences of some hydroxamic acids have recently been investigated by density functional and empirical methods.[51]

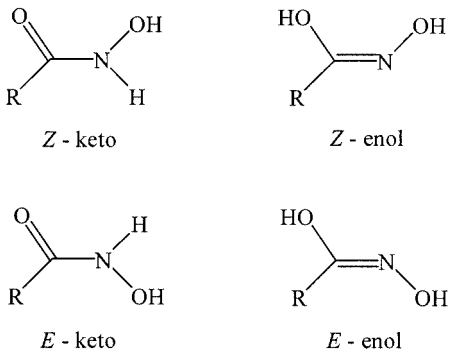



Figure 5. Tautomeric forms of hydroxamic acids

The acid−base equilibria and associated tautomeric forms have also been extensively described.[52−54] Semi-quantitative comparisons of the acidities of hydroxamic acids, amides and carboxylic acids have recently been reported.[55]

## 3. Complexation Behaviour

Hydroxamate ligands show a diversity of coordination behaviour that results in a rich array of metal complex structures. The complexing behaviour of hydroxamic acids and, in particular, aminohydroxamic acids has been comprehensively reviewed.[56]

### 3.1 Bidentate Coordination

It is hardly surprising that many biomedical applications of hydroxamic acids arise as a result of the strong chelating ability of the hydroxamate group. By far the most common mode of metal binding is through the deprotonated hydroxamate and carbonyl oxygen atoms (Figure 6), forming very stable five-membered chelates.

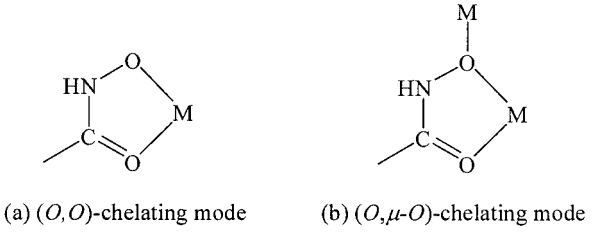



Figure 6. Modes of hydroxamate binding

The hydroxamato(1−) mode of coordination arises from the first deprotonation step involving coordination of the $NHO^-$ moiety. Further metal-induced deprotonation of the $NHO^-$ group leads to hydroximato(2−) complexes.[57]

Furthermore, the hydroxamate moiety possesses several sites for potential hydrogen-bond interactions with, for example, the backbone of enzymes, and these can be critical to their selectivity as enzyme inhibitors (Figure 7).

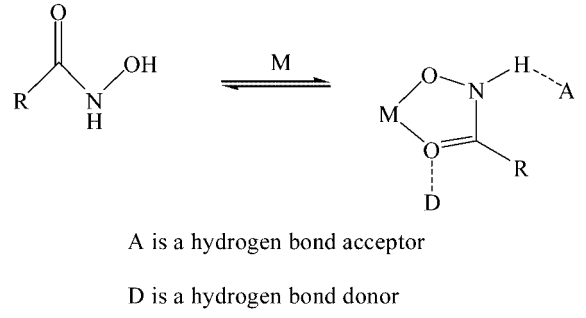



Figure 7. Sites for potential hydrogen-bonding interactions

Bidentate coordination of monohydroxamic acids to a number of transition-metal ions such as iron(III), cobalt(II) and nickel(II) generally results in the ready formation of 3:1 octahedral complexes,[58−60] while in the case of copper(II), 2:1 tetragonal complexes that have two additional monodentate ligands is the preferred geometry.[56,61]

### 3.2 Monodentate Coordination

Other modes of coordination, including monodentate binding through the deprotonated nitrogen or oxygen atom, have also been reported but these require specially designed coordination environments to provide additional stabilisation. The monodentate *N*-bonded trifluoroacetohydroxamate complex of carbonic anhydrase (a mimic of the hydrogen carbonate−enzyme product complex) is stabilised by hydrogen bonding with Thr-199 and by a weak F to $Zn^{II}$ interaction (2.8 Å) [Figure 8 (a)].[62] A similar complex of acetohydroxamate has also been characterised. The reaction of the octaethylporphyrin (OEP) complex Fe(OEP)Cl, which does not contain two adjacent vacant coordination sites to accommodate normal bidentate coordination, with benzohydroxamic acid gave [Fe(OEP)PhCONHO]·

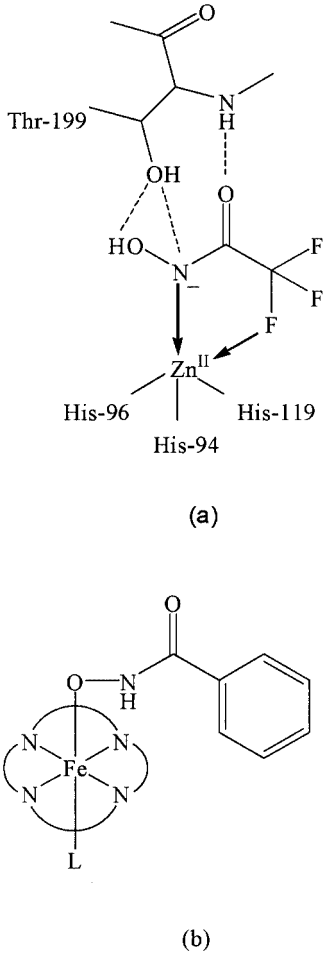



Figure 8. (a) Monodentate *N*-bonded trifluoroacetohydroxamate complex of carbonic anhydrase (a mimic of the hydrogen carbonate−enzyme product complex); (b) monodentate *O*-bonded benzohydroxamate in [Fe(OEP)PhCONHO]·PhCONHOH

PhCONHOH, which contains monodentate *O*-bonded hydroxamate stabilised by hydrogen bonding with free benzohydroxamic acid in the lattice [Figure 8 (b)].[63]

### 3.3 Hydroxamate Ligands with Other Coordinating Groups; Metallacrowns

The coordination behaviour of the hydroxamate group can be dramatically enriched by the incorporation of secondary coordinating groups at adjacent sites in the molecule. Dinuclear copper(II) complexes of α-aminohydroxamic acids that exhibit two modes of hydroxamate coordination (Figure 9), both giving five-membered rings, have been characterised. Under alkaline conditions, *N*,*N*- over *O*,*O*-coordination by copper(II) for α-aminohydroxamate ligands is preferred.[64,65]

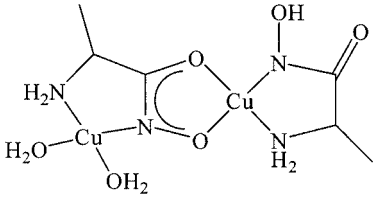



Figure 9. Two distinct bidentate binding modes observed in some complexes of α-aminohydroxamic acids

In the case of nickel(II), α-aminohydroxamate ligands produce bis *N*,*N*-bidentate square-planar complexes[59,66] rather than octahedral complexes containing *O*,*O*-bonded hydroxamates. With these ligands, the cobalt(III) complex forms tris complexes that have *N*,*N*-bidentate coordination.

The coordination properties of β-aminohydroxamic acids are, in general, similar to those of the α derivatives. Whilst β-aminohydroxamic acids can act as *N*,*N*-donors, this involves a six-membered ring, and *O*,*O*-bidentate coordination is generally preferred. A remarkable complex with the general formula $[Cu_5(LH_{-1})_4]$ was reported to be formed from the reaction of copper(II) salts with β-alaninehydroxamic acid (HL).[67] This complex, which is the major species in solution at pH 4.5−9, contains a twelve-membered ring made up of four metal ions linked in pairs by doubly deprotonated NO groups of hydroxamate ligands and another metal ion in a central cavity bonded by the four hydroxamate oxygen atoms. The amino nitrogen atom of one ligand and the carbonyl oxygen atom of another complete basal coordination around each outer copper ion, with the axial coordination of an oxygen atom (from water, perchlorate or a vicinal aminohydroximato ligand) completing the square-pyramidal coordination (Figure 10). This complex is the prototype for a remarkable family of compounds called metallacrowns subsequently developed by Pecoraro and co-workers.[68]

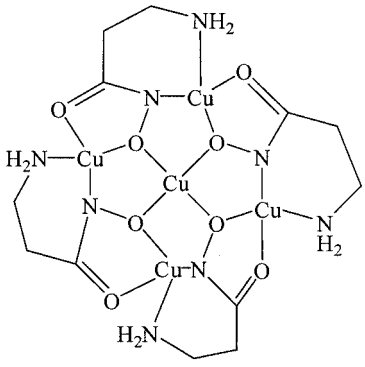



Figure 10. Structure of the copper(II) β-alaninehydroxamic acid complex displaying both *N*,*N*- and *O*,*O*-bidentate coordination through the hydroxamate

Following this, metallacrowns emerged as a new family of molecular recognition agents where hydroxamic acid ligands provided the structural support upon which the supramolecular metallacrown was constructed.[68−71] Salicylhydroxamic acid [Figure 11 (a)], which contains both hydroximato and phenolate donor atoms, is particularly well suited to the synthesis of metallacrowns because (i) three binding sites that are capable of stabilising high valent metal ions are available in the triply deprotonated ligand, and (ii) it is a dinucleating agent with all four heteroatoms as potential metal binding sites [Figure 11 (b)].

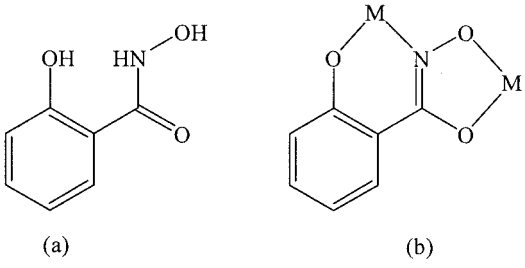



Figure 11. (a) Salicylhydroxamic acid; (b) salicylhydroxamic acid as a dinucleating agent with all four heteroatoms as potential metal binding sites

The ability of salicylhydroxamic acid to form a five-membered chelate ring with one metal through the hydroximate group and a six-membered ring to another metal through the substituted iminophenolate ring facilitates the formation of metal clusters with $M_A-N-O-M_B$ networks that link the ring metals [Figure 11 (b)]. The synthesis of most metallacrowns utilizes base to facilitate deprotonation of the ligand and counterions for charge balance in addition to metal salt and ligand.[68] Replacement of the phenolic group of salicylhydroxamic acid by an amino group affords a family of 2-aminophenylhydroxamic acid (anthranilic hydroxamic acid) metallacrowns. Other scaffolds such as 2-pyridylacetohydroxamic acid and 3-hydroxy-2-naphthohydroxamic acid have also been used in metallacrown synthesis. Although there is a change in charge from −3 to −2 on going from salicylhydroximato to anthranilic hydroximato

ligands, this does not have a pronounced impact on the resulting metallacrown structures but does result in the complex being uncharged. Recent work involving planar ligands such as picolinehydroxamic acid (picha) or nonplanar α-aminohydroxamic acids, e.g. glycinehydroxamic acid (glyha), afforded a new family of metallacrowns that are capable of accommodating a series of lanthanides in their cavities. The reaction of the appropriate hydroxamic acid with copper acetate and 1/5 equivalent of gadolinium(III) or europium(III) nitrates, for example, afforded $Gd(NO_3)_3$[15-MC$Cu^{II}$N(picha)-5], $Eu(NO_3)_3$[15-MC$Cu^{II}$N(picha)-5], and $Eu(NO_3)_3$[15-MC$_{Cu^{II}N(glyha)}$-5], all of which have been structurally characterized. Several other 15-metallacrown-5 complexes were synthesized with (i) $Cu^{II}$ or $Ni^{II}$ in the peripheral ring metal position, (ii) various lanthanides, $La^{III}$, $Nd^{III}$, $Sm^{III}$, $Eu^{III}$, $Gd^{III}$, Dy(III), $Ho^{III}$, $Er^{III}$, and $Yb^{III}$, encapsulated in the center of the ring, and (iii) chiral α-aminohydroxamic acids, e.g. phenylalaninehydroxamic acid, leucinehydroxamic acid and tyrosinehydroxamic acid as scaffolding. The complexes containing $Cu^{II}$ ions have the ring metals either in square-planar environments, bound to two hydroximato ligands, or in square-pyramidal geometries with solvent coordinated. The $Ni^{II}$ complexes appear to be either five- or six-coordinate. The encapsulated lanthanides are generally pentagonal bipyramidal, with five oxygen donors from the metallacrown ring, and solvent or bidentate nitrate ions in the axial positions. These complexes exhibit interesting magnetic behaviour due to the circular arrangement of ions. With Dy(III) encapsulated in the center of the ring, for example, a magnetic moment as high as 10.9 BM is achieved.[72]

Originally it was thought that metallacrowns could only be obtained from β-aminohydroxamic acids but a recent study has shown that in aqueous solution α-aminohydroxamic acids are also capable of forming metallacrowns, but these are less stable.[71]

### 3.3.1 Aminophenylhydroxamic Acid Complexes

As illustrated by the above structures the diversity of hydroxamate coordination modes can be enriched by the presence of secondary donor groups in the ligand. The influence of the position of these donors relative to the hydroxamate on the resulting structures is illustrated by the copper(II) complexes of isomeric aminophenylhydroxamic acids AphaH, which we recently reported.[73] The structures include a novel helical polymer formed by 3-AphaH, a dimeric copper(II)-metallacrown formed by 2-AphaH, and a simple mononuclear complex formed by 4-AphaH.

Reaction of $CuSO_4{\cdot}5H_2O$ with 3-AphaH in aqueous solution gave a helical polymer with the formula $[Cu_3(3\text{-Aphha})_4(H_2O)SO_4]_n{\cdot}8H_2O$ (Figure 12), the monomeric unit of which has three interlinked unique copper(II) sites. These are the neutral square-planar complex $Cu(3\text{-Apha})_2$, the anionic square-pyramidal complex $[Cu(3\text{-Apha})SO_4(NH_2)(\cdots NH_2)]^-$ and the cationic square-pyramidal complex $[Cu(3\text{-Apha})(H_2O)(NH_2)(\cdots NH_2)]^+$, where $NH_2$ represents amino groups from neighbouring molecules forming both normal and elongated (···) bonds. The Cu(2)−Cu(3) helical backbones are connected by $Cu^I$ square-planar sites. An extensive network of hydrogen bonds involving hydroxamate nitrogen and oxygen atoms, sulfate oxygen atoms, and both coordinated and lattice waters link the polymer chains. The copper(II) ions in this complex are magnetically independent.

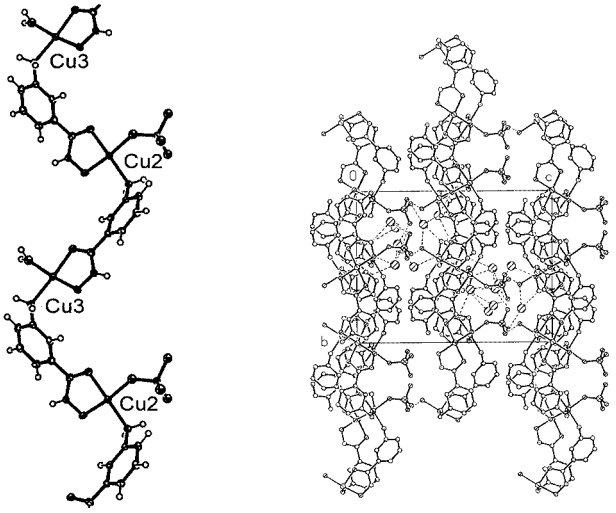



Figure 12. Molecular structure of $[Cu_3(3\text{-Aphha})_4(H_2O)SO_4]_n{\cdot}8H_2O$

The dimeric copper(II)-metallacrown complex $[Cu_5(2\text{-AphaH}_{-1})_4(\mu\text{-}SO_4)(H_2O)_2]_2{\cdot}10H_2O$ (Figure 13) was obtained by reaction of $CuSO_4{\cdot}5H_2O$ and 2-AphaH in aqueous solution. In this case, each monomeric unit contains a square of copper(II) ions, Cu1−4, linked by doubly deprotonated hydroximate ligands $2\text{-AphaH}_{-1}$ and a central copper(II) ion Cu5, which is coordinated by four hydroxamate oxygen atoms and the oxygen atom of a bridging sulfate that is also weakly bonded to Cu4. Pecoraro et al. reported a similar monomeric metallacrown structure, synthesised from $Cu(OAc)_2$.[74] The structure of $[Cu_5(2\text{-AphaH}_{-1})_4(\mu\text{-}SO_4)(H_2O)_2]_2{\cdot}10H_2O$, however, differs from that reported by Pecoraro et al. It is a fused dimeric metallacrown that has a novel "clam-like' structure, the closed end of which arises from the binding of ring copper atoms Cu2 and Cu2A in two metallacrown units to oxygen atoms O2A and O2, respectively, in adjacent units, and the open end of which accommodates bridging sulfate ligands. The external faces of the metallacrown dimer are involved in hydrogen bonding with other metallacrown units through hydroxamate nitrogen and oxygen atoms, resulting in intermolecular Cu−Cu bond lengths of approximately 3.9 Å. The complex is strongly antiferromagnetic. The only other reported dimeric metallacrown is that of a nickel(II) complex in which the metallacrown rings are parallel.[75]

In contrast to the above structures, reaction of 4-AphaH with $CuSO_4{\cdot}5H_2O$ resulted in the formation of a simple mononuclear complex $Cu(4\text{-Apha})_2{\cdot}H_2O$ (Figure 14). An extensive hydrogen-bonding network leads to an overall stacked structure containing internal cavities.

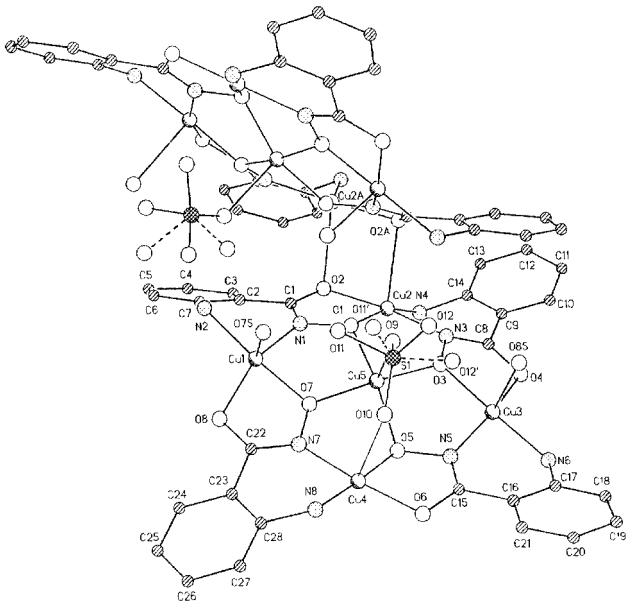


Figure 13. A novel dimeric copper(II)-metallacrown type complex $[Cu_5(2\text{-}AphaH_{-1})_4(\mu\text{-}SO_4)(H_2O)_2]_2\cdot 10H_2O$

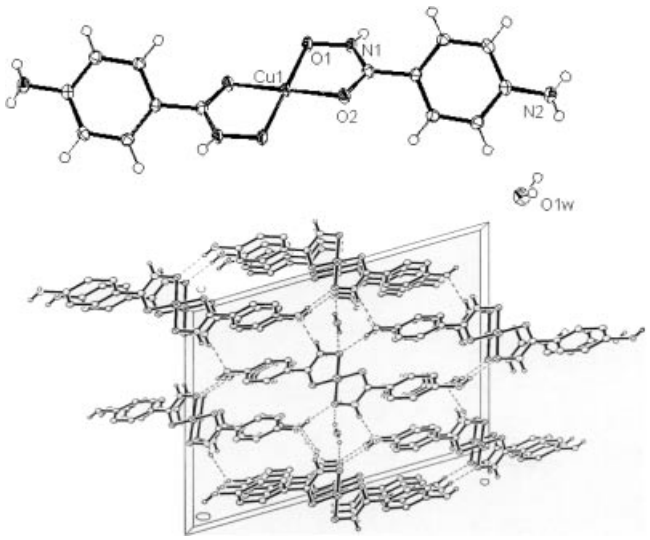


Figure 14. Mononuclear $Cu(4\text{-}Apha)_2\cdot H_2O$ exhibiting an extensive hydrogen-bonding network that leads to an overall stacked structure, which contains internal cavities

An intriguing heptanuclear nickel(II) complex that uniquely exhibits four distinct hydroxamate binding modes, two of which are novel, and which shows both antiferromagnetic and ferromagnetic interactions has also recently been reported by us.[76] Reaction of 2-(dimethylamino)-phenylhydroxamic acid (2-dmAphaH) with $NiSO_4\cdot 6H_2O$ results in the formation of $[Ni_7(2\text{-}dmAphaH_{-1})_2(2\text{-}dmApha)_8(H_2O)_2]SO_4\cdot 15H_2O$ (Figure 15), which contains a trigonal-bipyramidal array of nickel(II) ions with another two nickel(II) ions annexed to the apical sites. This complex in which each nickel ion is octahedrally coordinated contains monodeprotonated 2-dmApha and doubly deprotonated 2-dmAphaH$_{-1}$ hydroxamate ligands.

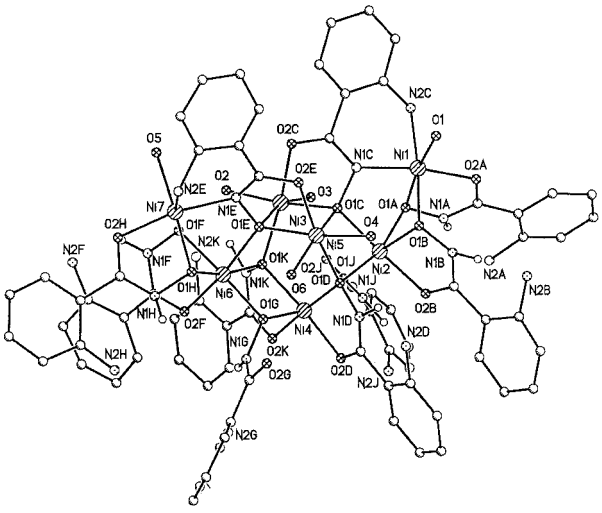


Figure 15. Molecular structure of $[Ni_7(2\text{-}dmAphaH_{-1})_2(2\text{-}dmApha)_8(H_2O)_2]SO_4\cdot 15H_2O$

The hydroxamate binding modes may be categorised as follows. One of the doubly deprotonated hydroxamate ligands C (Figure 15−17) is doubly bidentate with respect to nickel ions, Ni1/Ni3 when all of its donor atoms are involved, and is triply bridging to Ni2/Ni5/Ni3, when the hydroxamate oxygen atom is involved (Figure 17). Ligand E is similarly bonded to Ni5/Ni7 and Ni6/Ni3/Ni5. The remaining eight hydroxamate ligands are singly deprotonated and are coordinated through the hydroxamate group only. Ligand D is bidentate with respect to Ni4 and triply bridging through the hydroxamate oxygen atom with respect to Ni4/Ni2/Ni5. Ligand K is similarly coordinated to Ni4 and Ni4/Ni6/Ni3. Ligand G bridges Ni4 and Ni6 through the hydroxamate oxygen atom, while ligand J is similarly coordinated to Ni2 and Ni4. The four remaining hydroxamate

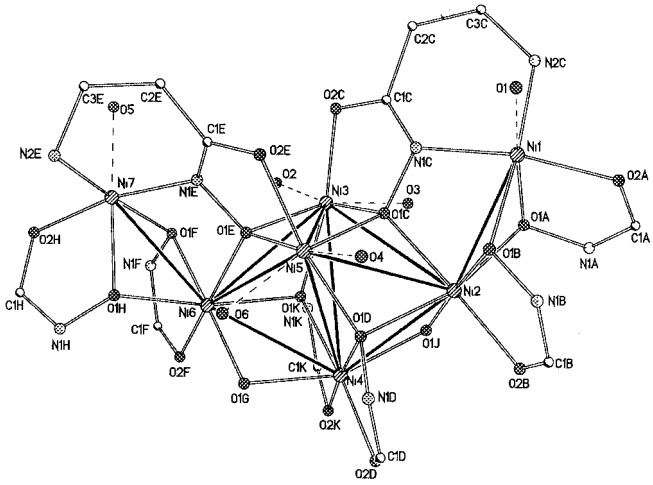
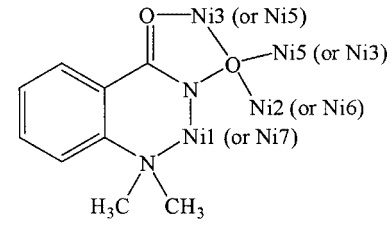


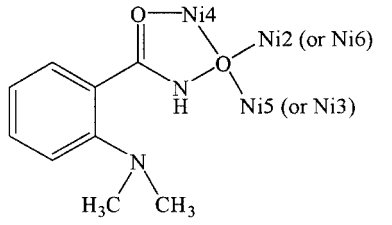
Figure 16. Molecular structure of $[Ni_7(2\text{-}dmAphaH_{-1})_2(2\text{-}dmApha)_8(H_2O)_2]SO_4\cdot 15H_2O$ illustrating the trigonal-bipyramidal arrangement of the five central $Ni^{II}$ ions, and an additional ion annexed to each apex

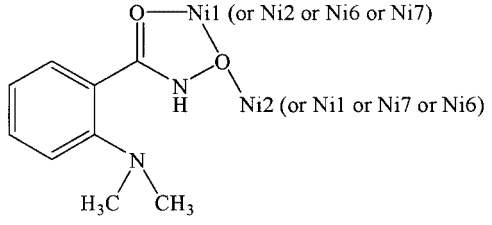
ligands coordinate pairs of Ni ions in a bidentate bridging manner. Ligands A and B are bidentate with respect to Ni1 and Ni2, respectively, and both ligands bridge these Ni ions through the hydroxamate oxygen atom. Ligands F and H exhibit the same coordination towards Ni6 and Ni7, respectively. The two binding modes observed for ligands D/K and G/J had not previously been reported for hydroxamate ligands, whilst a similar binding mode for ligands C/E, which involves an additional coordinating group, had only once previously been reported (for a nickel(II) metallacrown with salicylhydroxamic acid).[77] The double hydroxamate bridging of Ni1−Ni2 and Ni6−Ni7 by ligands A and B and F and H, respectively, is similar to that observed in some dinickel model systems for urease inhibition,[28,78] which also contain two hydroxamate bridges and for which inter nickel distances of 3.016 and 3.005 Å have been reported. These values are very similar to the values of 2.958(3) and 2.944(2) Å in the present case.



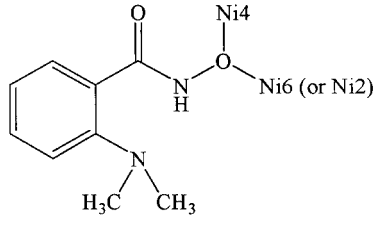

C (or E)




D (or K)




A (or B or F or H)




G (or J)

Figure 17. Binding modes of hydroxamate ligands (2-dmApahaH$_{-1}$ and 2-dmApha) in [Ni$_7$(2-dmAphaH$_{-1}$)$_2$(2-dmApha)$_8$(H$_2$O)$_2$]-SO$_4$·15H$_2$O

In contrast to the nickel product, reaction of 2-dmAphaH with CuSO$_4$·5H$_2$O gave the metallacrown [Cu$_5$(2-dmAphaH$_{-1}$)$_4$(HSO$_4$)$_2$(MeOH)$_2$]·2MeOH.[68] The presence of the methyl substituents preclude the formation of a dimeric metallacrown analogous to [Cu$_5$(2-AphaH$_{-1}$)$_4$(μ-SO$_4$)(H$_2$O)$_2$]$_2$·10H$_2$O, which we previously reported for 2-aminophenylhydroxamic acid (Figure 18).[73]

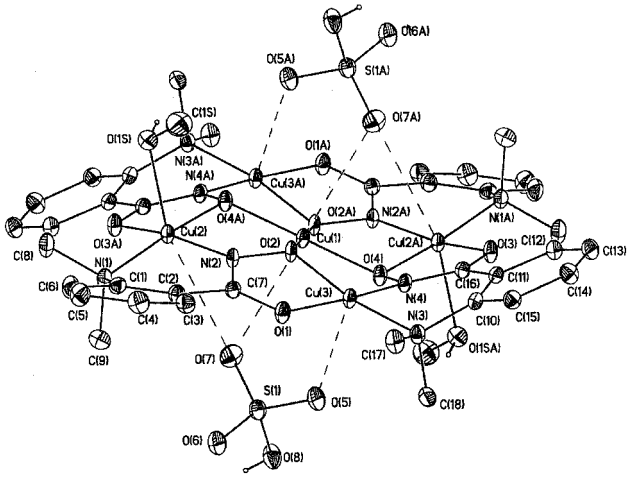



Figure 18. Molecular structure of [Cu$_5$(2-dmAphaH$_{-1}$)$_4$-(HSO$_4$)$_2$(MeOH)$_2$]·2MeOH

### 3.6 Hydroxamate Complexes of Ruthenium(III), Platinum(II), Palladium(II), Rhodium(I) and Rhodium(III)

Hydroxamate complexes of ruthenium(III), platinum(II) and palladium(II) were recently reported for the first time.[79−81] The fact that no ruthenium(III) hydroxamates had previously been reported is surprising in view of the extensive library of known iron(III) hydroxamates, and this is probably due to nitrosyl abstraction from hydroxamic acids by ruthenium(III) to give ruthenium(II)-nitrosyls as described in section 4. We have recently reported the structure of the first ruthenium(III) hydroxamate complex Ru(H$_2$edta)(2-OMepha) (Figure 19) from the reaction of NH$_4$[Ru(Hedta)Cl] with 2-methoxyphenylhydroxamic acid (2-OmephaH), and the synthesis and characterisation of several other complexes.

Upon addition of base, the hydroxamate ligand in this complex undergoes further deprotonation to give the hydroximato complex [Ru(edta)(2-OMephaH$_{-1}$)]$^{3-}$, Equation (2).

$$[Ru(edta)(2\text{-}OMephaH)]^{2-} \rightleftharpoons [Ru(edta)(2\text{-}OMephaH_{-1})]^{3-} \quad (2)$$

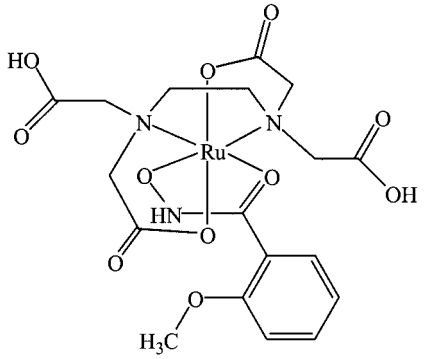


Figure 19. Structure of Ru($H_2$edta)(2-OMepha)

The formation of hydroximato complexes, although common in metallacrown chemistry, has been reported in only a few cases for mononuclear complexes.[82−84] The affinity of phenylhydroxamic acid for $[Ru^{III}(edta)(H_2O)]^-$ (hexacoordinate) was found to be much greater than for $[Fe^{III}(edta)(H_2O)]^-$ (heptacoordinate), but this was largely due to differences in charge and coordination numbers of the immediate metal coordination environments rather than intrinsic affinity differences between ruthenium(III) and iron(III) for hydroxamate ligands. The stability of ruthenium(III)-hydroxamate complexes is dependent on the substituent on the aryl ring.

Hydroxamic acid complexes of platinum(II) and palladium(II) have also recently been reported. It was revealed by $^1$H NMR spectroscopic studies that salicylhydroxamic acid (shaH) does not coordinate to chloro complexes of platinum(II) but can form complexes of the type $[Pt(shaH_{-1})(PPh_3)_2]$, the crystal structure of which was determined. In this complex, $shaH_{-1}$ binds through an (*O*,*O*) coordination mode in its hydroximate form, and the phenolic group remains protonated. Stabilisation of hydroximate binding to platinum is facilitated by the presence of soft ligands. In contrast, the palladium complex $[Pd(sha)_2]$ is readily synthesized and crystallizes as $[Pd(sha)_2](DMF)_4$, with the unexpected (*N*,*O*) binding mode of the salicylhydroxamate ligand.[80] Two novel platinum(II)-diamine benzohydroxamate complexes were reported, namely $[\{Pt(en)\}_2(\mu\text{-bha})]ClO_4\cdot H_2O$ (en = ethane-1,2-diamine, bha = benzohydroxamic acid) (Figure 20), and $[\{Pt(RRchxn)\}_2(\mu\text{-bha})]\cdot 2H_2O$ (chxn = cyclohexane-1,2-diamine) in which Pt−C bonds are present in the dinuclear structures. The two Pt centres are linked through the bha via (*O*,*O*) and (*C*,*N*) coordination modes, the latter involving formation of a Pt−C(*ortho*) bond. Both dinuclear complexes are less cytotoxic than their corresponding dichloro parent complexes. While the mechanism of action of the dinuclear species remains unclear, it has been suggested that the platinum(amine) moieties affect the activity but the presence of the hydroxamate bridge alters the rate of aquation and DNA binding of these species.[81] From these studies, it appears that only aryl hydroxamates readily bind to $Pt^{II}$. Furthermore, the hydroximate form of the ligand in these complexes appears to be stabilized by the binding of the second platinum centre bridged through the benzohydroximato ligand.

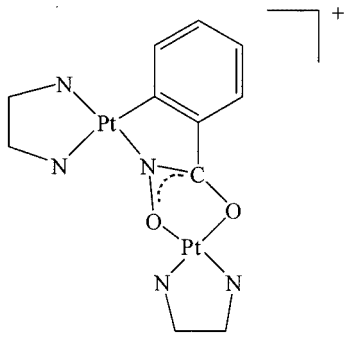


Figure 20. Structure of $[\{Pt(en)\}_2(\mu\text{-bha})]ClO_4\cdot H_2O$ (en = ethane-1,2-diamine, bha = benzohydroxamic acid)

A recent study involving the reaction of *N*-phenylbenzohydroxamic acids with rhodium showed that the oxidation state of the metal ion dictated the type of reaction involved. For rhodium(I), the *N*-phenylbenzohydroxamic acids serve as oxidizing agents and oxidise rhodium(I) to rhodium(III), and are themselves reduced to the corresponding amides. In the presence of rhodium(III), the same hydroxamic acids undergo typical bidentate hydroxamate coordination.[85] Interestingly, an unusual transformation of *N*-arylbenzohydroxamic acids, mediated by osmium(II), affords a family of cyclometalates in which the transformed hydroxamic acids are coordinated as doubly deprotonated benzanilides of osmium(III).[86]

## 4. Hydroxamic Acids as Nitric Oxide Donors

While some of the physiological roles of hydroxamic acids are undoubtedly due to the chelating ability of the hydroxamate group, others such as their hypotensive effects[33] (a known nitric oxide property) may be due to their ability to release nitric oxide (NO), a view strengthened by the now established importance of NO in many physiological processes. In fact, it is now well recognised that there are probably few pathological conditions where NO does not play an important role.[87−89]

Ruthenium(III) readily forms nitrosyls and there are more known nitrosyl complexes of ruthenium than any other metal.[90] The Ru−NO bond is generally very stable and is easily detected by infrared spectroscopy, ν(NO) at about 1880 $cm^{-1}$. We have recently shown that hydroxamic acids are effective NO donors by virtue of the fact that they readily transfer NO to ruthenium(III) forming highly stable ruthenium(II)-nitrosyls and by their ability to cause vascular relaxation of rat aorta by NO-mediated activation of the iron(II) haem-containing enzyme guanylate cyclase.[91] In doing so, we effectively used ruthenium(III) as a tool to abstract NO from hydroxamic acids. A similar reaction of hydroxamic acids with $K_3[Fe(CN)_6]$ to give $[Fe(CN)_5NO]^{3-}$ was reported independently at the same time.[92]

Reaction of the well-characterised $K[Ru(Hedta)Cl]\cdot 2H_2O$ with benzohydroxamic acid in aqueous solution at room temperature resulted in the unambiguous formation of a ruthenium nitrosyl complex. The microanalysis, IR, mass and $^1H$ NMR spectra are consistent with the formation of $K_2[Ru(edta)(NO)Cl]$, containing a linear, diamagnetic $Ru^{2+}-NO^+$ group (distinctive $\nu_{NO}$ at 1880 $cm^{-1}$) and no absorption at 1730 $cm^{-1}$, which confirms fully deprotonated edta. This complex is similar to the previously reported six-coordinate $Ru(H_2edta)(NO)Cl\cdot 2H_2O$,[93] but contains fully deprotonated tetradentate edta with two pendant carboxylate groups. A related complex $[Ru(edta)NO]^-$ was formed in solution by reaction of NO with K[Ru(Hedta)Cl].[94] Similar reactions of $K[Ru(Hedta)Cl]\cdot 2H_2O$ with acetohydroxamic acid and salicylhydroxamic acid also gave the same product in high yields. The products of the denitrosylation reactions were shown to be the corresponding carboxylic acids, Equation (3).

$$[Ru(Hedta)Cl]^- + RCONHOH \rightarrow [Ru(edta)(NO)Cl]^{2-} + RCOOH \quad (3)$$

Reaction of $RuCl_3\cdot xH_2O$ with aceto-, benzo-, salicyl- and anthranilic hydroxamic acids in ethanol, followed by purification on Sephadex LH 20 columns also gave ruthenium(II) nitrosyl complexes, all of which have very distinctive $\nu_{NO}$ bands at approximately 1885 $cm^{-1}$.

The ability of hydroxamic acids to release nitric oxide in simple biological systems was shown by vascular relaxation of endothelium-denuded rings (to remove endogenous nitric oxide) of rat aorta. These were set up in organ baths for isometric tension recording. Rings were contracted with the α-1-adrenoreceptor agonist phenylephrine (1μM), and the ability of increasing concentrations of hydroxamic acid derivatives to produce relaxation was examined (the results are shown in Figure 21). Of the hydroxamic acids investigated, benzohydroxamic acid proved most effective, causing approximately 45% relaxation of rat aorta at a concentration of 300 μM. Although this value is higher than that quoted for the well-known NO donor 3-morpholinosydnonimine, SIN-1 (1 μM), it compares favourably with that of another NO donor 4-(3-butoxy-4-methoxybenzo)-2-imidazolidilone, Ro-20−1724 (100 μM). Relaxation occurred by activation of the enzyme guanylate cyclase (a definitive receptor for NO) as shown by the fact that methylene blue (10μM), a known inhibitor of this enzyme, prevented the relaxation (e.g. relaxation to 300μM acetohydroxamic acid: vehicle treated, 31.4 ± 10.9% relaxation; methylene blue treated, 6.4 ± 3.1% relaxation, $n = 4$, $P < 0.05$).

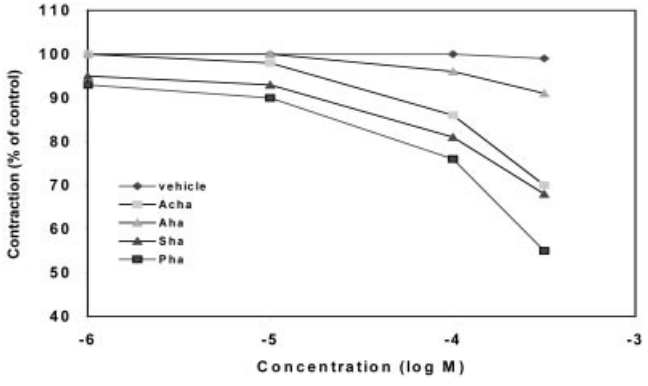



Figure 21. The effects of hydroxamic acids on relaxation of rat aorta

We have therefore shown conclusively that hydroxamic acids can act as effective NO donors by virtue of the fact that they can readily transfer NO to ruthenium(III). Furthermore, hydroxamic acids can cause vascular relaxation in rat aorta by NO-mediated activation of the iron-containing enzyme guanylate cyclase, thus confirming the biological relevance of our novel results.

## 5. Hydroxamic Acids as Inhibitors of Cyclooxygenase

Cyclooxygenase (COX) is the enzyme responsible for the generation of prostaglandins (Figure 22), bioactive lipids that are involved in many pathophysiological processes including vascular thrombosis, nocioception and temperature control.[95−97]

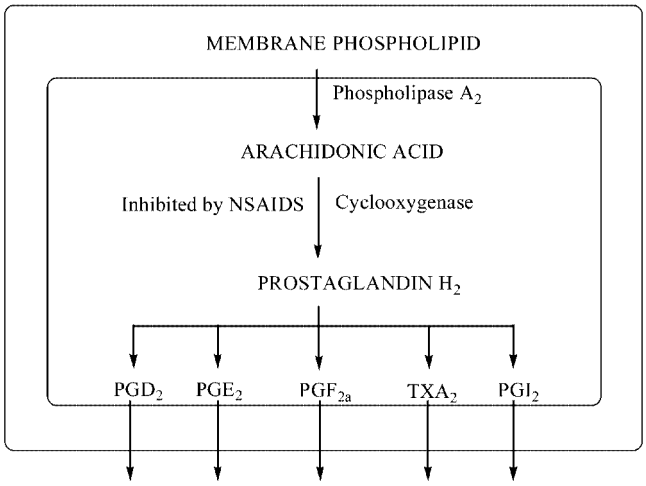



Figure 22. Production of prostaglandins by COX

Two isoforms of the enzyme have been identified: COX-1, which is expressed ubiquitously and is the only form in platelets where it generates thromboxane, a potent platelet activator; and COX-2, which was initially thought to be rare in normal tissue, but expressed at sites of inflammation, in tumours and in response to tissue injury. Both isoforms of COX are inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs)[98] such as aspirin, which were initially thought to have beneficial therapeutic effects due to COX-2 inhibition and side effects due to inhibition of COX-1. There has therefore been intense interest in the development of selective COX-2 inhibitors on the basis that prostaglandins that contribute to inflammation are produced by COX-2, while prostaglandins essential for normal

physiological processes are produced by COX-1. Several selective COX-2 inhibitors have resulted, including Celecoxib, Nimesulide and a range of modified NSAIDs such as aspirin analogues that have *o*-alkynyl ether or thioether substituents.[99] Aspirin (acetylsalicylic acid) is unique amongst the NSAIDs in that it covalently modifies the enzyme, irreversibly acetylating a serine residue in the substrate channel.[100]

Aspirin and other NSAIDS do not influence the peroxidase site of the enzyme. Therefore, after NSAID treatment, peroxidase (POX) activity can continue to generate damaging free radical species. There is therefore a need for dual COX and POX site inhibition. The hydroxamic acid Tepoxalin is such an inhibitor (Figure 23).[101]

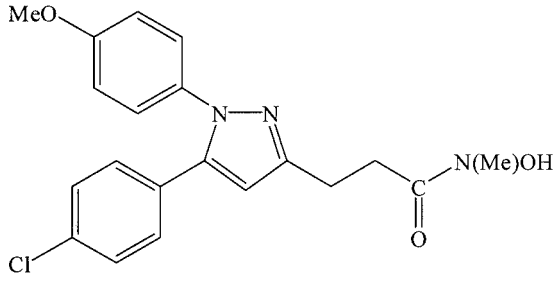



Figure 23. Structure of Tepoxalin

The rationale for the synthesis of selective COX-2 inhibitors has recently been thrown into doubt as it has been shown that COX-2 is expressed constitutively in many tissues and has important physiological roles, and that indeed COX-1 contributes to inflammatory processes. Therefore, it now appears that a combination of selective COX-1 and COX-2 inhibitors may possess greater efficacy than selective COX-2 inhibitors alone.[102]

We decided to investigate hydroxamic acid analogues of aspirin as COX inhibitors for the reasons that: (i) the hydroxamic acid group is less acidic than the carboxylic acid group and should cause less topical irritation; (ii) acetylated hydroxamic acid should, like aspirin, inhibit the COX site by acetylation of Ser-529; (iii) the acetylated hydroxamic acid should also inhibit the POX site since hydroxamic acids are known to inhibit peroxidases; (iv) since we showed that hydroxamic acids are potential NO donors, their aspirin analogues could behave like previously described NO-aspirins with anti-inflammatory effects but devoid of topical irritation, nonulcerogenic, and protective of the stomach.[103,104]

Acetylation of salicylhydroxamic acid with acetyl chloride afforded not the aspirin analogue but instead *O*-acetylsalicylhydroxamic acid, AcSHA, (Figure 24) in which the hydroxamic acid rather than the phenolic group was acetylated. This product was found, however, to be almost as potent as aspirin in the inhibition of cyclooxygenase and was found to require Arg-119 and Ser-529 for activity since the S529A and R119Q mutants were found to be inactive. A crystal structure of the inhibitor-COX-1 complex confirms that acetylation of Ser-529 had occurred, but that instead of the salicylhydroxamate metabolite being trapped in the active site, as was found in the case of bromoaspirin, a fresh molecule of inhibitor is found in this position. This is probably due to the fact that the active site pocket is positively charged and attracts the negatively charged acetylsalicylhydroxamate (i.e. a fresh molecule of inhibitor) with the exclusion of the neutral salicylhydroxamic acid. A mechanism of enzyme inhibition is shown in Figure 25.

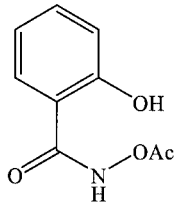



Figure 24. Structure of acetylsalicylhydroxamic acid

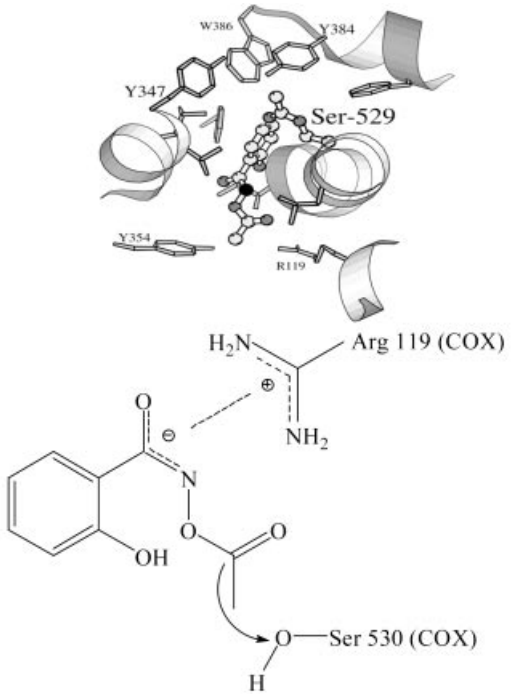



Figure 25. Structure of the COX-1 AcSHA complex, and the mechanism of inhibition of cyclooxygenase

Based on the results obtained with *O*-acetylsalicylhydroxamic acid (AcSHA), we synthesised triacetylsalicylhydroxamic acid (TriAcSHA) (Figure 26), and this was found to be much more effective than both aspirin (ASA) and AcSHA in inactivating both COX-1 and COX-2.[105,106] Hence, preincubation of COX-1 with inhibitor for 5 minutes yielded $IC_{50}$ values of 18 μM for TriAcSHA, and 60 μM for ASA. As with aspirin, mutation of serine-530 of COX-1 to an alanine abolished the activity of TriAcSHA. Mutation of arginine-119 to a glutamine markedly reduced the sensitivity towards TriAcSHA, therefore suggesting that this residue was necessary for interaction with the enzyme. Tri-

AcSHA was also more effective than aspirin as an inhibitor of platelet aggregation induced by arachidonic acid. The related diacetylated phenylhydroxamates, *N*-methyl-*O*,*O*-diacetylsalicylhydroxamic acid, *N*,*O*-diacetylbenzohydroxamic acid and 2-methyl-*O*,*N*-diacetylbenzohydroxamic acid showed reduced or absent activity against COX-1 (Figure 26). These data together suggest a mechanism of activity which involves Arg-119-assisted *N*-deacetylation of TriAcSha, followed by acetyl group transfer from the phenolate group of the inhibitor to Ser-529, possibly intramolecularly assisted by the anionic deacetylated nitrogen atom (Figure 26).

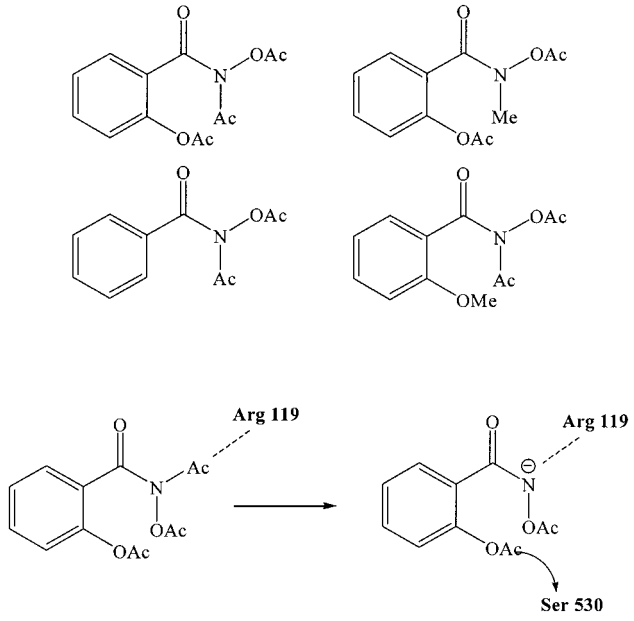



Figure 26. Acetylated salicylhydroxamic acid inhibitors of cyclooxygenase

We synthesized a series of triacylsalicylhydroxamic acids with progressively longer acyl groups (3−6 carbon atoms) (Figure 27). All of the compounds inhibited COX-1 and demonstrated progressively greater COX-1 selectivity with increasing number of carbon atoms (Figure 27). Hence, salicylhydroxamic acid provides a versatile backbone for the generation of a family of acylating inhibitors of COX.

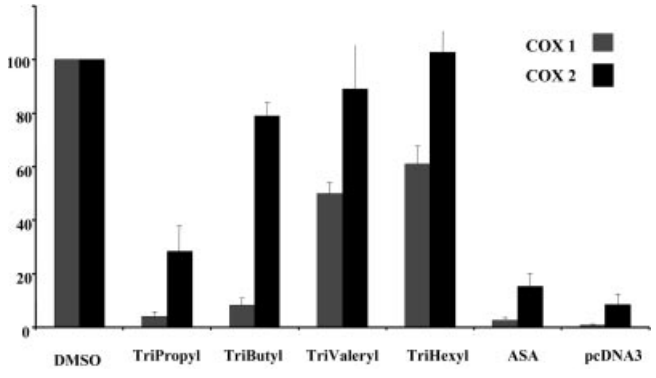



Figure 27. Triacyl derivatives of SHA are selective for COX-1

## 5. Conclusion

Hydroxamic acids are versatile ligands that give rise to a diverse range of metal complex structures, particularly if there are other metal binding groups present in the molecule. Their biomedical applications, once confined to treatment of metal overload conditions, have been extended to cover inhibition of a wide range of enzymes.

## Acknowledgments

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