Synthesis and charactrization of Imidazole Model Complexes of Inhibited Hydrolases

Majid Jary Muhamad
Chemistry Department, College of Science, University of Kufa

Abstract

Previous studies in this work showed that model dinuclear hydrolases of the general formula [M2(µ-H2O)(OAc)4(tmen)2]; where, M= Mn (Mn), Co, Ni reacted with hydroxamic acids to give a wide range of some hydroxamate bridged complexes whose structures mimic closely observed inhibited ureases. In veiw of the frequent occurrence of histidine as a ligating molecule in many metalloenzymes inlikely the hydrolases, it was decided to investigate the chemistry of a series of imidazole complexes analogous to the above models based on tmen as capping ligand.Model dinuclear hydrolases [M2(µ-H2O)(OAc)4(Im)4]; M= Ni (1), (2) and Co (3), prepared and fully characterized. Reaction of mono- and dihydroxamic acids with the model dinuclear hydrolases prepared above gave a wide range of some hydroxamate bridged complexes whose structures mimic closely observed inhibited ureases. For example, reaction of a monohydroxamic acid such as N-phenylacetohydroxamic acid [CH3C(=O)NC6H5OH], (N-PhAHA) with the dimeric complex [Ni2(H2O)(OAc)4(Im)4] gave the dark/green complex. [Ni2(OAc)(N-phAA)2(Im)4][OAc].2H2O and reaction of a dihydroxamic acid such as glutarodihydroxamic acid [(CH2)3{C(=O)NHOH}2.H2O], (GluH2A2) with the dimeric complex [Ni2(H2O)(OAc)4(Im)4] gave a Blue/green crystals of [Ni2(OAc){C(=O)N(O)(CH2)3C(=O)}(Im)4][OTF]2.In general, the above series based on the imidazole ligand was found to be susscceptable to easy dissociation in polar solvents such as methanol as approved by spectroscopic studies (IR, NMR, C.H.N. , elementary analysis and X-ray crystallography).

الخلاصة

بينـت الدراسـات السـابقة ان انزيمـات التحلـل المـائي موديـل ثنـائي النـواة للصـيغة العامـة [M2(µ-H2O)(OAC)4(tmen)2] عندما M = Mn,Co and Ni تتفاعل مـع حوامض الهايدروكزاميك لتعطـي مدى واسع من بعض معقدات الهايدروكزاميت الجسرية والتي تكون تراكيبها متشابهة تقريبا والتي لوحظ تثبيطهـا لانـزيم اليوريز .وبمراجعـة للظهـور المـالوف للهسـتدين كجزيئـة ارتبـاط لعـدة انزيمـات فلزيـة لاتشـبه انزيمات التحلل المائي ، تقرر البحث في كيمياء سلسلة من معقدات الايميدازول المشابهة للموديلات اعلاه اعتمادا على N,N,N,N-tetra methyl ethylenediamine (tmen) كليكاند غطائي .ان المعقدات ثنائي النواة [M2(µ-H2O)(OAC)4(Im)4] عندما M= Ni (1),(2),Co(3) تم تحضيرها وتشخيصها بشكل تام.اعطى تفاعل حوامض الهايدروكزاميك الاحادية والثنائية مـع انزيمات التحلل المائي موديل ثنـائي النواة اعلاه مدى واسع من بعض معقدات الهايدروكزاميت الجسرية والتي تكون تراكيبها متشابهة تقريبا والتي لوحظ تثبيطها لليوريزيز ،مثلا تفاعل حامض الهايدروكزاميك الاحادي N-فنيل اسيتو هايدروكزاميك مـع المعقـد ثنـائي الجزيئـة [Ni2(H2O)(OAC)4(Im)4] اعطـى معقـد اخضـر معـتم -N)Ni2(OAC)(N-phAA)2(Im)4][OAC].2H2O وتفاعل حامض الهايدروكزاميك الثنائي كليوتارو ثنائي الهايدروكزاميك مـع معقـد ثنـائي الجزيئـة[Ni2(H2O)(OAC)4(Im)4] اعطـى بلـورات خضـراء مزرقـة مـن

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[Ni2(OAc){C(=O)N(O)(CH2)3C(=O)}(Im)4][OTF]2 وبصورة عامة وجد ان السلسة اعلاه المعتمدة على ليكاند الايميدازول قابلة للتفكك بسهولة في المذيبات القطبية كالميثانول كما اثبتت عن طريق الدراسات الطيفية (الاشعة تحت الحمراء،الرنين النووي المغناطيسي ، التحليل الكمي العنصري الدقيق والتركيب البلوري عن طريق اشعة اكس).

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1. Introduction

The inhibition of metalloenzymes is of interest especially because of possible therapeutic applications. For example, the inhibition of matrix metalloproteinases by various hydroxamate-based inhibitors generally involves chelation of a mononuclear zinc centre by the hydroxamate function [1]

In order to understand the inhibition of urease enzyme by competitive inhibitors such as hydroxamic acids, fluoride ion, phosphate, thiols, diamidophosphate DAP, etc. dinuclear model complexes were prepared and reacted with inhibitors. Hydroxamic acids such as N-phenylacetohydroxamic acid (N-PhAHA) are potent inhibitors of urease and so were chosen for study in present work. A characteristic feature of hydroxamic acids is their ability to act as bidentate complexes with transition metal ions via the carbonyl and hydroxyl oxygen atoms [2].

1.1. Urease Model Inhibitors

Hydroxamic acids are potent inhibitors of urease [3] and the structure of the acetohydroxamate-inhibited Cys319Ala variant of Klebsiella aerogenes urease (KAU) shows the deprotonated hydroxy oxygen of the hydroxamic acid bridging the two nickel centres [4] in contrast to normal(O,O) chelation of a metal ion by a hydroxamic acid. A similar structure is found in the dizinc metalloenzyme Aeromonas proteolytica aminopeptidase Zn2AAP inhibited by p-iodo-D-phenylalanine hydroxamic acid [5]

Hydroxamic acids are also important bioligands and provided the first structural details of ligand binding to the dinickel active site of urease in which the acetohydroxamic acid used its deprotonated(OH) oxygen to bridge the two nickel ions and the carbonyl oxygen to bind to one nickel in a chelating/ bridging mode. A series of Ni(II) complexes of substituted hydroxamic acids with polymeric and tetrameric structures have been reported [6], whilst monosubstituted alkyl mono- and dihydroxamic acids coordinate to Ni (II) via the (O,O) coordination [7] and to form monomeric octahedral chelate complexes. [8-9].

1.2. Crystallographic Studies of Inhibition of Urease

Several X-ray crystal structures have been reported for the inhibition of urease, for example: Bacillus pateurii urease (BPU) inhibited with (β-ME) β-mercaptoethanol (HS-CH2CH2-OH) [10], while the structure and binding mode of this inhibitor to the active site of BPU has been described [11]. The structure of a KAU mutant urease Cys319Ala complexed with the competitive inhibitor acetohydroxamic acid AHA has also been reported [4] together with the structure of BPU inhibited with acetohydroxamic acid [12] and BPU inhibited with DAP diamidophosphate (NH2)2PO2 , [13]. The refined model of the β-ME inhibitor indicates a chelate binding mode through the terminal (OH) group, coordinated to Ni(1) and the (S) atom bridging the two nickel atoms in a bridging/chelating mode of binding (Figure

1), both Ni ions in β-ME-inhibited BPU appear to be penta-coordinated. The coordination geometry around Ni(1) is distorted square-pyramidal, and the coordination geometry of Ni(2) distorted trigonal bipyramidal.

The equatorial planes of the square planar and trigonal pyramids are joined through the bridging(S) atom of β-ME. The metal ion separation decreases upon β-ME binding from 3.7Å in the native enzyme to 3.1Å for β-ME-inhibited BPU.
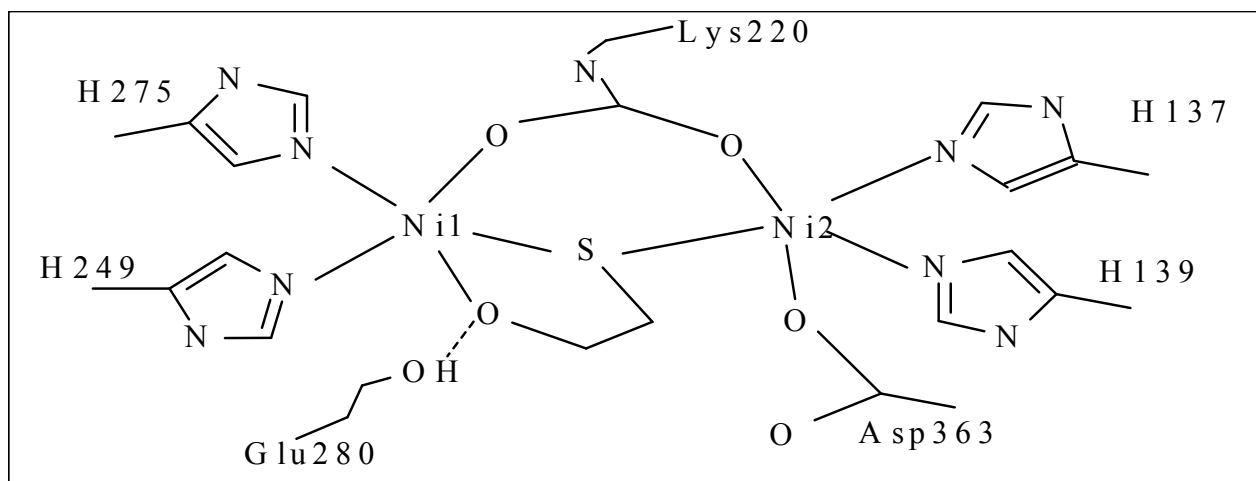

Figure 1: Model of the active site of BPU-inhibited with β-ME, showing the replacement of four water molecules in the native enzyme by the inhibitor.

2. Experimental

2.1 Materials

Reagent and solvents were used as obtained from commercial sources, without further purification unless otherwise stated. Methanol, dichloromethane, ethyl acetate, ethanol, diethylether and petroleum ether were dried by standard procedures. Nickel(II) acetate tetrahydrate (98%), Cobalt(II) acetate tetrahydrate (98%), urea (99.5%),

N,N,N,N-tetramethylethylenediamine (98%), trimethylsilyltrifloromethanetriflate (TMS-OTf) (99%), hydroxylamine hydrochloride (99%), were purchased from Aldrich Chemical Company Limited, London.

2.2 Instrumentation

(C.H.N)Elementary analysis, were performed by the microanalytical laboratory, Chemical Services Unit, University College Dublin. Infrared spectra were recorded in the solid state 1-2% KBr (potassium bromide) discs and solution in a calcium fluoride cell, path length 0.1mm using a Perkin-Elmer FT-IR Paragon 1000. 'H NMR spectra were obtained at 300MHz on a Jeol-GX 300MHz spectrometer using a range of deuterated solvents with TMS as a reference and 5.05mm tubes (for diamagnetic species) and 5.28, 5.35mm tubes (for paramagnetic species). 13C NMR spectra were obtained similarly and all spectra were recorded at 30ºC (all NMR s. UV/visible spectra were recorded on a ATI Unicam UV/vis Spectrometer.

2.3 Preparation of Mono and Dihydroxamic acids (HA`s)

Monohydroxamic acids can be prepared by several methods, such as the modification of Blatt`s procedure and generally involve the reaction of an ester

with hydroxylamine [14]. This reaction is carried out in alcoholic solution in presence of equimolar quantities of a base such as sodium alkoxide or sodium carbonate. Other methods involve oxidation of aldoximes, amines, amides and nitriles, and also reactions between aldehydes and sodium hydroxamate [15]. However, these methods generally result in a formation of several side products, thus lowering the yield of the hydroxamic acid obtained and complicating purification procedures. It is important to exclude water from the reaction mixture as the amide bond of the hydroxamic acid is susceptible to hydrolysis, which results in several side products. Finally, salts (for example, KCl or NaCl) are the major side product produced in these preparations which influence the purity of the hydroxamic acids prepared

2.3.1: Preparation of Monohydroxamic acids
2.3.1.1: Preparation of N-phenylacetohydroxamic acid
[CH3C(=O)NC6H5OH], (N-PhAHA)
Step 1: Preparation of N-Phenylhydroxylamine

20ml (0.2 mol) of nitrobenzene was added dropwise with vigorous stirring to a solution of 15g of zinc dust in a 1:1 mixture of methanol/water, (c.a. 90ml), in a 500ml three necked round bottomed flask, 3.5ml of a saturated solution of ammonium chloride in deionized water was then added dropwise (the temperature reaches c.a. 40°C and continuoe rising to 60-65°C with addition of drops of ammonium chloride solution. It is important not to allow the temperature to rise over 65°C, as this results in a markedly reduced yield. The temperature was maintained for one hour either by intermittent use of an ice bath or by warming on an oil bath. The solution was filtered (Buckner) and the remaining zinc oxide washed with 200ml of boiling water, and 150g of NaCl added to the filtrate. The solution was then cooled in an ice bath for two hours until pale yellow needles formed which were filtered and the filtrate discarded, 80ml of diethyl ether were added and the solution was dried by repeated addition of MgSO4. The mixture was filtered, decanted into a weighed 250ml flask, and evaporated to produce pale cream crystals of N-phenylhydroxylamine (Figure 2). The crystals were dried at an oil pump and used immediately as they are unstable in both air and light (M.A. Table 1).Yield: (10.9g, 10mmol, 50%).It is important to use accurate equivalent numbers of moles here based on the yield in step 1.
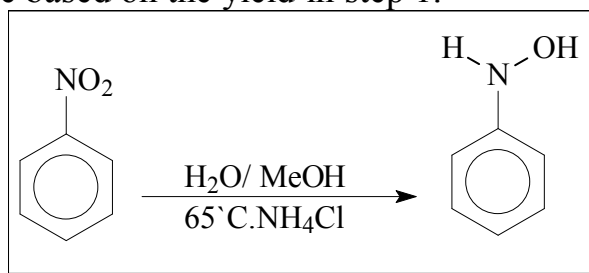

Figure 2. Preparation of N-phenylhydroxylamine

Step 2: Preparation of N-phenylacetohydroxamic acid

To a solution of N-phenylhydroxylamine 10.9g (0.1mol) in 100ml of diethyl ether, 10.9g (0.15mol) of NaHCO3 were added, stirred and cooled to −8°C in an ice-salt

bath. Acetyl chloride 5.7ml (0.1mol) in 20ml of diethyl ether was added dropwise over one hour. The solution was filtered, evaporated in vacuum to give a golden oil, which was triturated with a 10% solution of NaHCO3 for 10 minutes; the mixture was then filtered and dissolved in 150ml of hot ethanol, stirred with activated dry charcoal (5g) and stirred for one hour on a warming plate (50ºC), filtered and evaporated to obtain a pale yellow oil which was chromatographed on a silica gel column and eluted with 1:2 (petroleumether/ethylacetate). The eluant was evaporated and the residue dissolved in ethyl acetate and chromatographed again on an alumna gel column, and again an oil was obtained which was further purified by stirring in NH3(aq) for 15 minutes until the pH reached 9. On cooling and addition of dilute H2SO4 until the pH reached 4, a pale yellow oil was obtained which was kept in a refrigerator for two days to give a light yellow solid. Recrystallization from hot ethyl acetate gave colourless crystals of N-phenylacetohydroxamic acid (Figure 3). (M.A. Table 1) Yield: (8.5g, 60mmol, 56%).
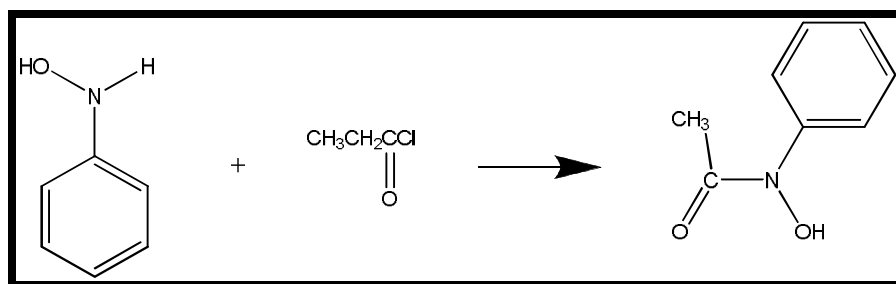

Figure 3. Preparation of N-phenylacetohydroxamic acid

2.3.2 Preparation of Dihydroxamic acids

2.3.2.1 Preparation of Glutarodihydroxamic acid [(CH2)3{C(=O)NHOH}2.H2O], (GluH2A2)

Step 1

Under nitrogen, 6.606g (0.05mol) of glutaric acid was added to a stirred solution 16.22g (0.1mol) of 1,1-carbonyldiimidazole in 150ml of dried THF, and the solution stirred overnight. When the reaction was completed, evolution of CO2 ceased and the mixture was then filtered and the solid dried completely at the pump, washed with 50ml of diethylether, and returned to the reaction vessel. A solution of 0.1mole of free hydroxylamine in methanol was prepared by the addition of 5.6g (0.1mol) of KOH to 6.9g (0.1mol) of hydroxylamine hydrochloride in methanol (small amount of alcohol) and filtered by suction under Nitrogen to remove the resulting KCI.

Step2

Under nitrogen, the solution of free hydroxylamine was added slowly to the glutarodiimidazole powder, which was then stirred overnight, glutarodihydroxamic acid precipitates as fine white solid. Continued stirring increases the yield of isolated product.The product was recrystallized from 1:1 acetone/water mixture to give a saturated solution (on reflux) which was left to slowly cool, and on standing gave a pure crystalline product.(M. A. Table 1). Yield (5.98g, 63%).

The carbonyldiimidazole does not need to be completely dissolved in THF prior to the addition of the carboxylic acid.A minimum amount of alcohol should be used in the preparation of the free hydroxylamine (c.a. 20ml).Number of moles for step 1 must be calculated in order to use the equivalent moles for the next stepTo prepare a compound substituted at the N or O position, the appropriate hydroxylamine should be used. It can be added to the diimidazole solution in THF with a small volume of alcohol (10ml). This causes the imidazole hydrochloride to precipitate from the solution when cooled. The solution was then filtered and the solvent removed. The resulting oil was extracted for several times with hot ethyl acetate and on cooling and/or the addition of ether the glutarodihydroxamic acid (Figure 4) will precipitate, alternatively the substituted hydroxylamine is added as in the main preparation, the product did not precipitate from the alcohol solution due to its increased solubility but can be recovered by removal of alcohol and extraction with hot ethyl acetate as above.
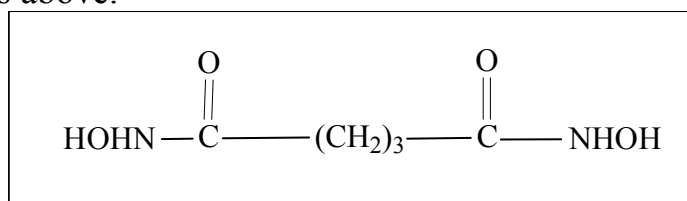
$$HOHN - \overset{\overset{\displaystyle O}{\|}}{C} - (CH_2)_3 - \overset{\overset{\displaystyle O}{\|}}{C} - NHOH$$

Figure 4. Preparation of Glutarodihydroxamic acid

Table 1. Microanalytical data for Hydroxamic Acids.

Compound		%C	%H	%N
CH3CON(C6H5)OH	Calc.	63.56	6.00	9.26
(N-PhAHA)	Found	63.47	5.98	9.17
HON(H)C(=O)(CH2)3C(=O)N(H)OH. H2O	Calc.	33.33	6.71	15.55
(GluH2A2)	Found	33.39	6.50	15.47

Table 2. Infrared spectroscopic data (cm−1) for hydroxamic acids

Hydroxamic acids	υ(N-O)	υ(C-N)	υ(C=O)	υ(O-H)
CH3CON(C6H5)OH (N-PhAHA)	954	1526	1628	2916
HONH(CH2)3NHOH (GluH2A2)	944	1551	1655	3023

2.3.3. Preparation of the Dinuclear Model Hydrolases containing Imidazole (Im) ligands

2.3.3.1. Preparation of [Ni^2(H$_2$O)(OAc)^4(Im)$_4$], (1)

2.48g (10mmol) of [Ni(OAc)2.4H2O] were dissolved in 50ml of methanol, filtered and a solution of 1.63g (24mmol) of imidazole in 15ml methanol was added dropwise over 15 minutes with stirring and the volume of solvent reduced and diethylether was slowly diffused into the solution to precipitate a dark/green solid. (M.A. Table 3). Yield (2g, 6.2mmol, 62%).

2.3.3.2. Preparation of [Co(OAc)2(Im)2], (2)

2.48g (10mmol) of [Co(OAc)2.4H2O] were dissolved in 150ml of methanol, which was filtered and a solution of 1.63g (24mmol) of imidazole in 20ml methanol was

added dropwise for 15 minutes with stirring for 12hour, after filtration, the solvent was reduced under vacuum and left for slow diffusion of diethylether into the solution. Dark/purple crystals of [Co(OAc)2(Im)2] were obtained over a week suitable for crystallographic analysis. (M.A. Table 3) Yield (2.7g, 7.2mmol, 73%)

2.3.3.3. Preparation of [Co2(H2O)(OAc)4(Im)4], (3)

2.48g (10mmol) of [Co(OAc)2.4H2O] were dissolved in 150ml of a 1:1 water/acetonitrile mixture, refluxed to give a clear solution, which was filtered and a solution of 1.63g (24mmol) of imidazole in 20ml of acetonitrile (CH3CN) was added dropwise over 15min with stirring for 12hours. after filtration and when the solution was left standing in a wide necked flask to complete dryness, two products physically separated, a dark purple product, [Co(OAc)2(Im)2], (2) and a light pink [Co2(H2O)(OAc)4(Im)4], (3). Recrytallization from deionized water gave crystals of both products, which were washed in diethylether, and dried.

Yield of dimer (3) (2.1g, 6.2mmol, 62%) (M. A. Table 3),

Yield of monomer (2) (0.081g, 2mmol, 21%) (M. A. Table 3)

(Avoid warm the crystalline solusion).

Table 3. Elemintary analysis data for dinuclear (Im) complexes

Compound		%C	%H	%N
[Ni2(H2O)(OAc)4(Im)4] (1)	Calc.	37.30	4.69	17.40
	Found	37.06	4.59	
	17.11			
[Co(OAc)2(Im)2] (2)	Calc.	38.35	4.50	17.89
	Found	38.18	4.43	17.90
[Co2(H2O)(OAc)4(Im)4] (3)	Calc.	38.27	4.69	17.39
	Found	38.46	4.93	19.46

2.3.4. Preparation of hydroxamate dinuclear (Im) complexes

2.3.4.1. Preparation of the Dibridged N-Phenylacetohydroxamate, Dinickel Complex [Ni^2 (OAc)(N-PhAA)$_2$(Im)$_4$][OAc]. 2H$_2$O, (4)

0.643g (1mmol) of compound (1) was dissolved in 50ml of MeOH, the solution was filtered, and a solution of N-PhAA 0.302g (2mmol) in 5ml MeOH was added, stirred for 15minutes, filtered and solvent removed under vacuom to give an oil which was extracted with 3x50ml of 1:1 mixture diethylether/n-hexane to give the dark/green complex. [Ni2(OAc)(N-PhAA)2(Im)4][OAc].2H2O.

Yield: (0.5g, 68 mmol, 68%) (M.A. Table 4)

2.3.4.2. Preparation of the Deprotonated N-hydroxyglutarimide, Dinickel Complex, [Ni^2(OAc){C(=O)N(O)(CH2)3C(=O)}(Im)$_4$][OTF]$_2$, (5)

Under nitrogen, a solution of (1) 0.643g (1mmol) in 50ml of dried MeOH, TMS-OTF (0.36ml, 2mmol) was injected and the solution was stirred for 1 hour. A solution of previously refluxed hot glutarodihydroxamic acid (GluH2A2) 0.223g (1.25mmol) in 10ml of dried MeOH was added and stirred for 30 minutes, filtered and the solvent removed under vacuum to give an oil which was dissolved in 10ml hot acetonitrile, layered with a 1:1 mixture diethylether/cyclohexene (c.a. 3x50ml) to deposit blue/green crystals of (1,GluH2A2)

Yield: (0.26g, 59 mmol, 60%) (M.A. Table 4)

## Table 4. Elemintary analysis data for dibridged hydroxamate (Im) dimers

Compound	%C	%H	%N
[Ni2(OAc)(N-PhAA)2(Im)4][Oac].2H2O (4)	Calc. 45.50 Found 44.98	5.03 4.58	16.58 15.81
[Ni2(OAc){O(N)(O=C)2(CH2)}(Im)4] [OTF]2 (5)	Calc. 28.83 Found 29.89	2.86 3.38	14.41 15.41

3. Results and Discussion:

As mentioned above, previous studies of model inhibited hydrolases such as urease were based upon the dinuclear complexes, [M2(OAc)4(H2O)(tmen)2], where M= Co(II) and Ni(II) and a study of their reactions with both urea and a range of hydroxamic acids [16-17]. In this chapter, the chemistry of the analogous imidazole complexes is explored since these should be closer models to the hydrolases, which generally contain histidines as metal N-donor ligands.

3.1. Imidazole Based Model Complexes [Ni^2(OAc)$_4$(H$_2$O)(Im)$_4$], (1), [Co^2(OAc)$_4$(H$_2$O)(Im)$_4$], (3) and [Co(OAc)$_2$(Im)$_2$], (2).

Complex (1) was prepared as dark/green crystals by the reaction of a solution of [Ni(OAc)2.4H2O] in methanol with a 2.4 ratio excess of imidazole also in methanol for 15 minutes and subsequent work-up (see experimental section 2.3.3.1). Satisfactory microanalysis was obtained (Table 3) and UV/visible and infrared spectra are given (Tables 6 and 7). In the case of complex (3), the analogous reaction in methanol always gave the monomeric complex [Co(OAc)2(Im)2], (2), but the analogous reaction in an acetonitrile/water 1:1 mixture gave as major product the pink dimeric complex [Co2(OAc)4(H2O)(Im)4], (3), and smaller quantities of the dark purple monomer [Co(OAc)2(Im)2], (2). These complexes were formed at the same time from recrystallization in deionised water and the crystals were physically separated and purified (see experimental sections 2.3.3.2 and 2.3.3.3).

[Co(OAc)2.4H2O] + Im → monomer (2) + dimer (3)
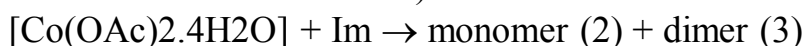
The crystal structure of [Co(OAc)2(Im)2], (2) has been reported [18] and proves that (2) is a monomer. Similar structure of the closely related monomeric series, [M(RCOO)2(2-X-Im)2]; M= Zn, Co; R= Me, Et, Pr; X= Me, Et were reported nearly 20 years ago [19] but were not referenced in the above paper [18]. The position with regard to the corresponding dimers [Co2(OAc)4(H2O)(Im)4], M= Co, Ni, Mn is confusing. Metal-metal distances are quoted in reference [20] for the cobalt and nickel dimers but no structural or preparative details are given nor could any reference be found in a search of the (Cambridge Crystallographic Data Base); however, the crystal structure of the manganese dimer has been fully reported [21]. Finally, a crystal structure of the dicobalt complex [Co2(OAc)4(H2O)(Im)4], (3) (Figures 5 and 13) was obtained and confirmed the presence of a bridging water molecule and showed the similarity in dimeric structure of (3) to the (tmen) series [Ni2(OAc)4(H2O)(tmen)2], (B) and [Co2(OAc)4(H2O)(tmen)2], (D). However, it

should be noted that our Co-Co distance of 3.3546Å is quite different from that of reference [20].

The formation of both (3) and (2) in the same reaction suggests that (3) readily dissociates (see Lippard et al [22] for the formation of complex A) and that this behaviour may be solvent dependent and indeed this is the case as shown below by the following IR spectroscopic studies.
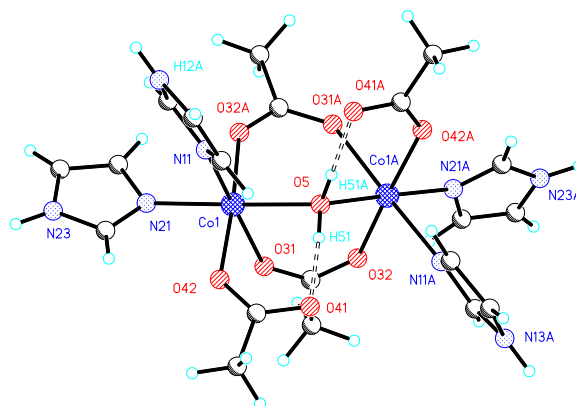

Figure 5. X-Ray Crystal Structure of [Co2(H2O)(OAc)4(Im)4], (3)

3.1.1. Spectroscopic Studies
3.1.1.1. UV/visible spectra

The electronic spectra of both (1) and (3) in methanol were shown in Table 6. The nickel complexe (1) showed three bands in the region (981- 1075), (637- 389) and (373- 389)nm being assigned to the three spin-allowed transitions 3A2g(F)→ 3T2g(F); 3A2g(F)→ 3T1g(F); and 3A2g(F)→ 3T1g(P) for an octahedral d8 complexes, whilst the cobalt(II) complexe (3) showed two broad bands in the regions (933- 1089) and (528- 575)nm, assigned to two spin-allowed transitions, the first band is assigned to the 4T1g→ 4T2g transition and the absorption in the visible region to the 4T1g→ 4A2g and 4T1g→ 4T1g(P) transitions [23]. Finally (2) showed bands at 541 and 1096nm in accord with the near octahedral structure reported [18] in the crystal structure.

Table 5. Crystal data and structure refinement for complex[Co2(H2O)(OAc)4(Im)4], (3)

Crystal Data	
Empirical formula	$C_{20}H_{30}Co_2N_8O_9$
Formula weight	M = 644.38
Temperature	180(2) K
Wave length(lambda)	0.71073 A
Crystal system	Orthorhombic
space group	Aba2
Unit cell dimentions	a = 8.6346(9), alpha = 90 o
	b = 16.6711(18), beta = 90 o
	c = 18.991(2) A , gamma = 90 o.

Volume (U)	**2733.7(5) A3**
	(by least squares refinement on 6239 reflection positions),
Crystal dimensions	**0.48 x 0.44 x 0.08 mm,**
F(000)	**1328.**
Z	**4**
Density (calculated)	**1.566 Mg/m3**
Absorption coefficient{mu(MoK-alpha)}	**1.276 mm-1.**
Crystal character:	**block block.**
Maximum theta	**32.86o.**
The hkl ranges	**-11/ 7, -25/ 24, -22/ 22.**
8326 reflections measured, 3304 unique	**[R(int) = 0.0381].**
Absorption correction by	**Semi-empirical from equivalents;**
Minimum and maximum transmission factors:	**0.58; 0.93.**
Space group	**Aba2 or Cmca (alternative axis setting).**

3.1.1.2. Infrared Spectra of (1) and (3) complexes in Solid State (KBr)

The infrared spectra of (1) and (3) complexes (Table 7) showed carbonyl peaks at (1635, 1615, 1630, 1611) cm-1 respectively, assigned to bidentate bridging acetates and (1542, 1538, 1533, 1537) cm-1 assigned to the monodentate bridging acetates. Each complex showed two identical weak broad peaks at (2023, 2111), (1942, 2055), (2024, 2088) and (2018, 2120)cm-1 respectively, assigned to the bridging water. These two water peaks are diagnostic in assigning (1) and (3) as dimers.

The monomeric complex $[Co(OAc)_2(Im)_2]$, (2) (Figure 6) exhibits a medium-intensity, broad band at 3420cm-1 (present in complex 3), assigned to ν(N-H) and the broadness is indicative of hydrogen bonding, in accord with the crystal structure [18]. Two $\nu_{as}(CO_2)$ (1593,1573) cm-1 and two $\nu_s(CO_2)$ at (1419, 1403) cm-1 bands were observed for (2), indicating that there are two different geometries of the acetate groups with two different types (syn and anti) of hydrogen bonds connecting the imidazole groups in the solid state. If the hydrogen bonding is considered as weak, the acetate groups can be regarded as two types of bridges, namely tri- and mono-atomic. So the bands at 1593 and 1403cm-1 were assigned to the stretching modes of the monoatomic acetate bridges, and those at 1574 and 1419cm-1 to the triatomic acetate bridges. Owing to the more asymmetrical bonding of the monoatomic carboxylate group, a large splitting (Δ 190cm-1 in ½D) of the CO2 stretching frequencies was observed [24].
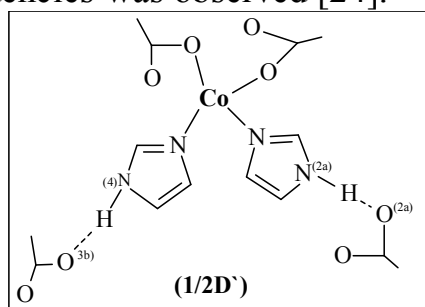

Figure 6. Structures $[Co(OAc)_2(Im)_2]$, (2)

The crystal structures of the homologous compounds $[Co(RCOO)_2(2\text{-Me-Im})_2]$, (Figure 7) reported [19] showed that for R= CH3 and C3H7 the complexes

crystallized as 6-coordinate structures, while for R= C2H5 a 4-coordinate structure results. These findings suggest that there is very little free energy difference between the 4- and 6-coordinate structures. Presumably minor changes in crystal-packing forces as the carboxylate alkyl chain length is varied account for the variability in coordination number in the crystalline state.
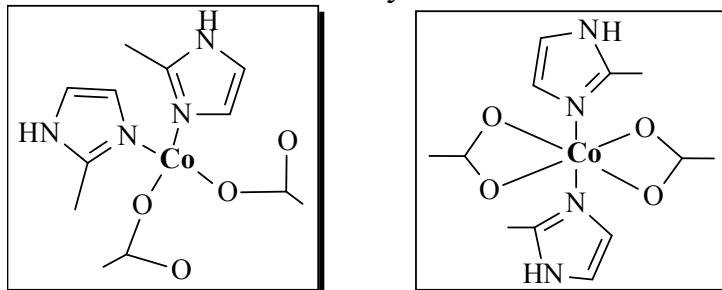

Figure 7. The complex [Co(RCOO)2(2-Me-Im)2] in 4-coordinated (Left) and 6-coordinated (Right)

The 6-coordinate complex reverts to 4-coordinate structures upon dissolution in ethanol. The shift in the band position to lower energy upon dissolution was clearly evident. In contrast, the solution-state and solid-state spectra are virtually identical with complexes which are 4-coordinate in the solid state. This isomerization process is also corroborated by infrared evidence. The symmetric and asymmetric C-O stretching of the carboxylate moiety are separated by about 190cm-1 in the known 4-coordinated complexes both in the solid state and in solution. In the crystalline 6-coordinate complexes this separation decreases to about 100cm-1, diagnostic of bidentate coordination by the carboxylate groups [25], when the 6-coordinate complexes are dissolved in alcohol, this separation goes up to about 190cm-1, indicative of monodentate coordination by the carboxylate moieties in solution. In summary, these structural studies show a novel type of linkage isomerism [19].

Table 6. Uv/vis spectroscopic data for dinuclear model hydrolases containing (Im) ligands

Medium	(1)	(3)
UV/vis λmax.nm E.cm-1.M-1	391(35) 653(17) 1082(7)	549(27) 953(-3)

Table 7. Infrared spectroscopic data (cm–1) for dinuclear model hydrolases containing (Im) ligands

Medium	(1)	(3)	Assignment
KBr	1615 1538 1942, 2055	1611 1538 2018, 2120	Bidentate bridging acetate Monodentate bridging acetate Bridging water

3.2. Reactions of (1) and (3) with Monohydroxamic acids

3.2.1. Reactions of (1) and (3) with N-Phenylacetohydroxamic acid (N-PhAHA)

The imidazole dibridged hydroxamate complex [Ni2(OAc) (NPhAA) 2(Im)4] [OAc] .2H2O , (4) (Figure 8) was obtained as a blue/green solid by the above method by reacting [Ni2(H2O)(OAc)4(Im)4], (1) with N-phenylacetohydroxamic acid (N-PhAHA) in a 1:2 ratio in methanol but in the absence of KPF6 and after subsequent work-up, satisfactory microanalysis was obtained (Table 4). Infrared and UV/visible spectra are given in (Tables 8 and 9)
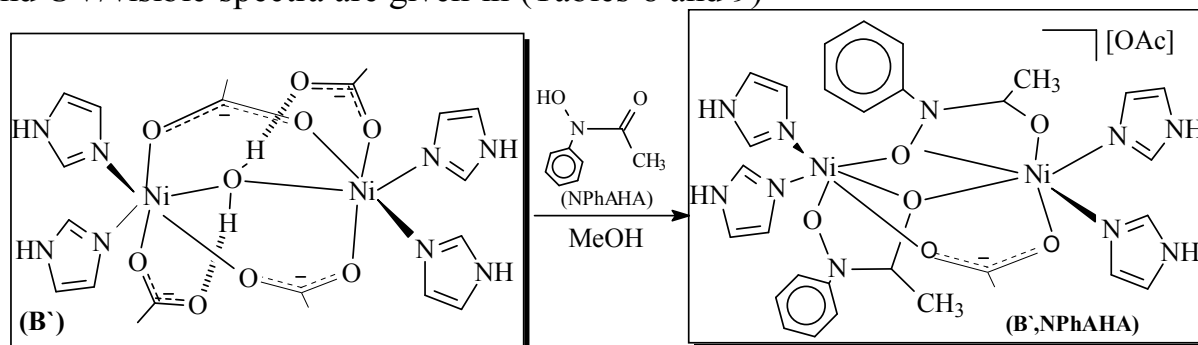

Figure 8. Complex [Ni^2(OAc)(NPhAA)$_2$(Im)$_4$][OAc].2H$_2$O, (4) from the reaction of (1) with (N-PhAHA)

3.2.1.1. Spectroscopic Studies

3.2.1.1.1. UV/visible Spectra

The complex (4) was measured in methanol and are typical of distorted octahedral Ni(II) complex. Thus, (4) showed three bands in the region (980-981), (637-643) and (313-373)nm (Table 8).

3.2.1.1.2. Infrared Spectra in Solid State (KBr)

The infrared spectrum of the dibridged (4) in the carboxylate region show peaks at (1609, 1606), assigned to the bidentate bridging acetates and bridging hydroxamates (Table 8).

3.3. Reactions of (1) and (3) with Dihydroxamic acids

3.3.1. Reactions of (1) and (3) with Glutarodihydroxamic acid

[Ni2(OAc){O(N)(O=C)2(CH2)3}(Im)4][OTf]2 , (5) was prepared as green/blue crystals by the reaction of a solution of (1) in methanol with TMS-OTf and glutarodihydroxamic acid (GluH2A2) in methanol (see experimental section) and gave crystals suitable for X-ray structural analysis. Satisfactory microanalysis were obtained (Table 4) and infrared and UV/visible spectra are given in (Table 9).

3.3.1.1. Spectroscopic Studies

3.3.1.1.1. UV/visible Spectra

The electronic spectrum of (5) is typical of distorted trigonal-bipyramidal d8 Ni(II), which showed two bands for penta-coordination at 641 and 381nm could be assigned to d-d transitions of 3B1(F)→3E(F) and 3B1(F)→3A2, 3E(P), respectively [35-36] (Table 9), and the spectra also showed broad band at 1061nm

3.3.1.1.2. Infrared Spectra in Solid State (KBr)

The infrared spectrum of (5) complex showed infrared peaks in the carbonyl region at (1618, 1575), assigned to bridging acetates and coordinated hydroxamates,

respectively, additional peaks at (1684 cm-1) was assigned to the carbonyl of the bidentate glutarimide (Tables 8 and 13) respectively.

Table 8. Infrared spectroscopic data (cm–1) for hydroxamate inhibited-dinickel model hydrolases containing the (Im) ligand.

Medium	(4)	(5)	Assignment
KBr	1607 1591	1618 1575 1684	Bidentate bridging acetate Coordinated Hydroxamate Bidentate glutarimide

Table 9. Uv/vis spectroscopic data for dinuclear model hydrolases containing (Im) Ligands

Medium	(4)	(5)
UV/vis λmax.nm E.cm-.M-	381(8), 641(3), 1061(2)	373(41), 637(15), 981(10)

4. Conclusion

Model dinuclear hydrolases [M2(μ-H2O)(OAc)4(Im)4]; M= Ni (1), (2) and Co (3), prepared and fully characterized. In general, the above series based on the imidazole ligand was found to be susscepteable to easy dissociation in polar solvents such as methanol as improved by spectroscopic studies (IR, NMR, MS). In the case of cobalt complex, there is some confusion in the literature with a reported crystal structure of the monomeric [Co(OAc)2(Im)2] and a Co-Co distance of 3.687Å reported for the dimer [Co2(μ-H2O)(OAc)4(Im)4] apparently prepared by the same method as the monomer using methanol as solvent. We were unable to locate the details of this crystal structure on the Cambridge Data Base. However, using a mixed acetonitrile/water solvent and subsequent physical separation of the crystals, we obtained both the monomer and the dimer, and the crystal structure of the dimer is similar to that of the previously reported dimer [Co2(H2O)(OAc)4(tmen)4], with tmen as capping ligand with a bridging water molecule and the monodentate acetates intramolaculary H-bonded to the water as in the tmen complex but also intermolecularly H-bonded to the N-H of an imidazole of another dimer molecule. The Co-Co distance is 3.3546Å considerably different from that reported in reference [20]. The hydroxamate bridged complexes [M2(AA)2(OAc)(Im)4]+; M= Co, Ni, AA= deprotonated hydroxamate, were prepared for (1) and (3) respectively, and in all cases in methanol as solvent. Despite the dissociation of (1) and (3) in MeOH, the products were pure and again with spectroscopic properties confirming in all cases their dimeric nature and illustrating the ability of hydroxamic acids to induce dimerization when acting in the bridging bonding mode involving the

deprotonated hydroxamate oxygen as bridging the two metal centres and the ketonic oxygen bonding one metal centre only. Interestingly (3) and (1) react with glutarodihydroxamic acid in the same manner as [Co2(H2O)(OAc)4(tmen)4], with elimination of one hydroxylamine and formation of the dimmer [M2(OAc){O(N)(O=C)2(CH2)3}(Im)4][OTf]2; M= Co, Ni, involving bridging deprotonated N-hydroxyglutarimide but with only one bridging acetate and penta-coordinated Co(II) in contrast to the product with [Co2(H2O)(OAc)4(tmen)4]; [Co2(OAc)2{O(N)(O=C)2(CH2)3}(tmen)2][OTf], which contains two bridging acetates and hexa-coordinated Co(II) [17]. The similarity between the structures of these bridged hydroxamates and those reported for the inhibited enzymes gives support for the use of these models in attempts to ellucidate the inihibition mechanism for urease and related dinuclear metalloenzymes.

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