Available online at www.sciencedirect.com
SCIENCE DIRECT®
Polyhedron 22 (2003) 3255–3264
POLYHEDRON
www.elsevier.com/locate/poly

Reactivity of copper(II) peptide complexes with bioligands (benzimidazole and creatinine)

Ángel García-Raso [a,*], Juan J. Fiol [a,*], Bartomeu Adrover [a], Pere Tauler [b], Antoni Pons [b], Ignasi Mata [c], Enrique Espinosa [c], Elies Molins [c]

[a] *Departament de Química, Universitat de les Illes Balears, 07122 Palma de Mallorca, Spain*
[b] *Departament de Biologia Fonamental i Ciències de la Salut, Universitat de les Illes Balears, 07122 Palma de Mallorca, Spain*
[c] *Institut de Ciència de Materials de Barcelona, CSIC, Campus de la Universitat Autònoma, 08193 Cerdanyola, Spain*

Received 6 May 2003; accepted 17 July 2003

Abstract

The nature of the peptide seems to be a very important factor for the reactivity of copper(II) peptide complexes with bioligands. Thus, although we have tried to obtain all the ternary complexes derived from the dipeptides L-ala-gly, gly-L-tyr and gly-L-trp with benzimidazole and creatinine, only four new ternary Cu(II) peptide complexes have been obtained: [Cu(gly-L-tyr)(benzimidazole)]·H_2O (**1**), [Cu(L-ala-gly)(benzimidazole)]·$3H_2O$ (**2**), [Cu(gly-L-trp) (creatinine)]·$1.25H_2O$ (**4**) and [Cu(L-ala-gly)(H_2O)(creatinine)]·$2H_2O$ (**5**). Compounds **1**, **2** and **4** exist as slightly distorted square planar complexes with the four coordination sites occupied by the tridentate peptide dianion and a nitrogen of the ligand, while compound **5** present a square pyramidal co-ordination in which the axial position is occupied by a water molecule. In the ternary benzimidazole complexes the lateral chain of the peptide moiety seems to determine the relative orientation of the ligand. In contrast, in the creatinine complexes the presence of two important intramolecular hydrogen bonds, involving the exocyclic NH_2 and C=O groups of the creatinine molecule, yield a nearly coplanar system which is independent of the nature of the peptidic lateral chain. These compounds do not present catalase-like activity nor remarkable SOD-like activity but the values of IC_{50} permit to distinguish a different behavior between benzimidazole and creatinine ternary complexes. Thus, benzimidazole compounds show lower IC_{50} (similar to free Cu(II)) values than creatinine ones.

Keywords: Copper; Peptide; Creatinine; Benzimidazole; Bioactivity

1. Introduction

Ternary complexes formed between metal ions and two different types of bioligands, namely heteroaromatic nitrogen bases and amino acids (or peptides) may be considered as models for substrate–metal ion–enzyme interactions and other metal ion mediated biochemical interactions. Among these compounds, copper(II) complexes are known to play a significant role either in naturally occurring biological systems or as pharmacological agents [1–3].

A considerable number of X-ray crystallographic studies on glycylglycine-copper(II)–ligand (benzimidazole [4], creatinine [5], cytosine [6,7], isocytosine [8], methylisocytosine [8], cytidine [9,10], 9-methyladenine [11], 7,9-dimethylhypoxantine [12], 1,10-phenantroline [13], 2,9-dimethyl-1,10-phenantroline [14], imidazole [15], imidazolate [16] and 4,4′-dipyridyl [17,18]) have been reported. However, only ternary complexes with 1,10-phenantroline (phen): (L-tyr-gly)-copper(II)-(1,10-phenantroline) [19], [Cu(L-ala-gly)(phen)]·$3.5H_2O$ [20], [Cu(L-val-gly)(phen)] [20], [Cu(gly-L-trp)(phen)]·$2H_2O$ [20] and isocytosine: [Cu(ala-gly)(isocyt)(H_2O)]·H_2O [21] and [Cu(L-tyr-gly) (isocyt)]·$3H_2O$ [21] have been described.

Benzimidazole and creatinine, the ligand moieties of the ternary complexes presented in this work, are of

* Corresponding authors. Tel.: +971-173263; fax: +971-173426.
E-mail addresses: dquagr0@clust.uib.es (A. García-Raso), dqujfa0@clust.uib.es (J.J. Fiol).

0277-5387/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.poly.2003.07.013

considerable interest according to their biological and pharmacological properties.

Creatinine (2-amino-1,5-dihydro-1-methyl-4H-imidazol-4-one), the final metabolic product of creatine plays an important role in protein metabolism and its level in serum and urine is indicative of the renal function. The study of binary and ternary complexes of this bioligand should be of interest due to the fact that creatinine metabolism might be connected with its complexation to different metal ions [22]. Five co-ordination modes have been established by X-ray crystallography: (i) bidentate bridging through N(1)(ring) and the deprotonated exocyclic NH site [23]; (ii) bidentate binding via N(1)(ring) and the exocyclic O(C=O) [24]; (iii) monodentate binding through the N(1)(ring) site [25]; (iv) monodentate binding through the exocyclic O(C=O) [26]; and (v) monodentate fashion through the deprotonated exocyclic NH group [24].

Benzimidazole as the 5,6-dimethyl derivative is present in vitamin B_{12} and related biomolecules [27] and other benzimidazole compounds have found wide use as anthelmintic agents for both human and veterinary purposes [28]. In addition, it has been reported that several copper complexes with benzimidazole derivatives present inhibitory effects on helminth parasites [29] and more recently, benzimidazole riboside compounds like 5,6-dichloro-2-(isopropylamino)-1-β-L-ribofuranosyl-1H-benzimidazole are presented as a new class of antiviral compounds that are potent inhibitors of human cytomegalovirus (HCMV) replication [30].

On the other hand, it has been reported that certain copper complexes can exhibit superoxide dismutase activity [31]. This activity depends on the Cu(II)/Cu(I) redox process, which has been related to more or less flexibility on the geometric transformation around the metal centre [32].

In this context, we report on the preparation, the spectroscopic characterization, the crystal structures and several redox biological assays of four ternary complexes peptide–copper(II)–biological ligand, [Cu(gly-L-tyr)(benzimidazole)] · H_2O (**1**), [Cu(L-ala-gly)(benzimidazole)] · $3H_2O$ (**2**), [Cu(gly-L-trp)(creatinine)] · $1.25H_2O$ (**4**) and [Cu(L-ala-gly)(H_2O)(creatinine)] · $2H_2O$ (**5**). An improved procedure to prepare the previously described [Cu(gly-gly)(benzimidazole)] · $3H_2O$ (**3**) [6] has also been included.

2. Experimental

2.1. Physical measurements

Elemental analyses were carried out using a Carlo Erba model 1106 microanalyzer. The infrared spectra were registered in the solid state (KBr pellets) on a PE 683 and the electronic spectra on a PE 552 spectrophotometer. ESR spectra were recorded at X-band frequencies with a Bruker ESP-300E spectrometer at 298 K. Magnetic measurements were carried out on powdered samples with a pendulum-type magnetometer (Manics DSM8). For magnetic and ESR data see text (Section 3.4). Reagents (gly-L-ala, gly-L-val, gly-L-phe, gly-L-tyr, gly-L-trp, L-val-gly, L-ala-gly, benzimidazole and creatinine) were used as received from Aldrich.

2.2. Preparation of the complexes

The synthesis of these ternary complexes Cu(peptide)(benzimidazole or creatinine) strongly depends on the nature of the peptide moiety. In our hands we have explored the preparation of the corresponding ternary complexes with gly-L-ala, gly-L-val, gly-L-phe, gly-L-tyr, gly-L-trp, L-val-gly and L-ala-gly, modifying the reaction conditions (solvent, temperature, stoichiometry of the reagents, pH and reaction time). Unfortunately, these experiments were generally unsuccessful, yielding binary copper compounds. Only it has been possible to isolate ternary complexes with L-ala-gly (benzimidazole and creatinine), gly-L-tyr (benzimidazole) and gly-L-trp (creatinine).

2.2.1. Synthesis of [Cu(gly-L-tyr)(benzimidazole)] · H_2O (**1**)

To a 5 ml aqueous solution of $CuSO_4 \cdot 5H_2O$ (0.25 g, 1 mmol) and gly-L-tyr (0.24 g, 1 mmol) were added, while stirring, 2 ml of 1 N NaOH. To the resulting suspension, solid benzimidazole (0.12 g, 1 mmol) was added and the mixture was stirred for a few minutes. The pale blue precipitate was collected by filtering off and then dried in air (83%). A few suitable crystals for X-ray diffraction were grown from a solution obtained by addition of a few drops of DMF into a suspension of the crude material in methanol. (Found: C, 49.81; H, 4.33; N, 12.84. *Anal.* Calc. for $C_{18}H_{20}CuN_4O_5$: C, 49.60; H, 4.59; N, 12.86%.) IR (cm^{-1}): 300w, 325w, 366w, 429m, 531m, 553m, 583m, 624w, 640m, 654w, 718w, 747m, 766w, 779w, 814m, 857m, 889w, 933w, 989m(sp), 1018w, 1038m, 1098m, 1119m, 1135w, 1177m, 1220m, 1258m, 1285m, 1312m, 1387m(br), 1414m, 1440s, 1473m, 1512s, 1612vs(br), 3323m and 3628m(sp). UV–Vis (methanol): λ 616 (ε 81), 279 (7.1×10^3), 272 (7.0×10^3) and 225 nm (1.3×10^4 M^{-1} cm^{-1}). Λ_M/Ω^{-1} cm^2 mol^{-1} (10^{-3} M in methanol, 20 °C) = 16.4.

2.2.2. Synthesis of [Cu(L-ala-gly)(benzimidazole)] · $3H_2O$ (**2**)

A solution of L-ala-gly (0.15 g, 1 mmol) and $CuSO_4 \cdot 5H_2O$ (0.25 g, 1 mmol) in 1 N NaOH (2 ml) was added to a methanolic solution of benzimidazole (0.12 g, 1 mmol). The resulting solution was stirred at room temperature for ten minutes and then filtered. The blue solution obtained was allowed to evaporate slowly and

after several days violet needles were filtered and dried in air (24%). (Found: C, 37.60; H, 5.29; N, 14.72. *Anal.* Calc. for $C_{12}H_{20}CuN_4O_6$: C, 37.94; H, 5.27; N, 14.75%.) IR (cm^{-1}): 310m, 321m, 349w, 385w, 431m, 451m, 552m, 561m, 634w, 650w, 769s, 891m, 982s, 1010w, 1061w, 1095m, 1174w, 1194w, 1230w, 1261m, 1292m, 1306m, 1398m, 1425m, 1437m, 1474w, 1515m, 1585s(br) and 3283w. UV–Vis (water): λ 624 (ε 65), 278 (4.0×10^3), 271 (4.4×10^3) and 241 nm (6.0×10^3 M^{-1} cm^{-1}). Λ_M/Ω^{-1} cm^2 mol^{-1} (10^{-3} M in water, 20 °C) = 6.3.

2.2.3. Synthesis of [Cu(gly-gly)(benzimidazole)] · $3H_2O$ (**3**)

The binary complex aqua(glycylglycinato)copper(II) (0.11 g, 0.5 mmol), obtained from the method of Manyak et al. [33], in 5 ml of water was added to a solution of benzimidazole (0.06 g, 0.5 mmol) in 5 ml of methanol. The mixture was stirred and heated up to boiling and then allowed to cool at room temperature while stirring. The resulting suspension was filtered and after a few minutes the complex was obtained as violet needles in a noticeably higher yield (67%) than was reported before (ca. 10%) [6], Suitable crystals for X-ray diffraction were grown from a solution of DMA/H_2O (10/1). (Found: C, 36.14; H, 4.86; N, 15.23. *Anal.* Calc. for $C_{11}H_{18}CuN_4O_6$: C, 36.11; H, 4.92; N, 15.32%.) IR (cm^{-1}): 315w, 433w, 450w, 530w, 560w, 604m, 631m, 715m(br), 770m, 866w, 891w, 983m, 1036m, 1117w 1160w, 1259m, 1300m, 1399s, 1434m, 1450w, 1473w, 1514s, 1587s, 1603s, 1624m(sh) and 3300m. UV–Vis (ethanol): λ 610 (ε 98), 278 (7.8×10^3), 272 (7.4×10^3) and 242 nm (1.0×10^4 M^{-1} cm^{-1}). Λ_M/Ω^{-1} cm^2 mol^{-1} (10^{-3} M in ethanol, 20 °C) = 1.7.

2.2.4. Synthesis of [Cu(gly-L-trp)(creatinine)] · $1.25H_2O$ (**4**)

To a solution of $CuSO_4 \cdot 5H_2O$ (0.25 g, 1 mmol) in 10 ml of distilled water were added consecutively, while stirring, solid gly-L-trp (0.26 g, 1 mmol) and 2 ml of 1 N NaOH. To the resulting suspension, 1 mmol (0.113 g) of creatinine was added and the mixture was stirred at room temperature for five minutes. The dark blue precipitate obtained was separated by filtering off and then dried in air (48%). Suitable crystals for X-ray diffraction were grown after several days by dissolution of the product in hot water. (Found: C, 44.40; H, 4.89; N, 18.22. *Anal.* Calc for $C_{17}H_{20}CuN_6O_4 \cdot 1.25H_2O$: C, 44.53; H, 4.91; N, 18.34%.) IR (cm^{-1}): 309w, 327m, 369vw(br), 393w(br), 431m, 461vw(br), 485m, 531m, 573m, 606m, 633w, 655w, 693m, 729m, 756s(sp), 767s(sp), 811vw(br), 850w, 869w, 881vw, 893w, 938w(br), 969w, 981w, 1028m, 1048s(sp), 1100s, 1123m, 1152m, 1219w, 1236m, 1283s, 1340s(br), 1355s, 1369s(br), 1426s, 1503m(br), 1603vs(br), 1621s, 1684s, 1725w, 2844w, 2936w, 3285m(br) and 3343m. UV–Vis (water): λ 628 (ε 76), 281 (4.4×10^3) and 221 nm (3.3×10^4 M^{-1} cm^{-1}). Λ_M/Ω^{-1} cm^2 mol^{-1} (10^{-3} M in water, 20 °C) = 16.1.

2.2.5. Synthesis of [Cu(L-ala-gly)(H_2O)(creatinine)] · $2H_2O$ (**5**)

A sample of creatinine (0.056 g, 0.5 mmol) was added at room temperature to a water solution (5 ml) of the binary complex obtained by the reaction of freshly prepared $Cu(OH)_2$ and L-ala-gly according to the method of Manyak et al. [33]. The resulting solution was heated with stirring and maintained near the boiling point for 10 min. The solution was then kept at room temperature and a blue crystalline precipitate appeared after several days. The product was separated by filtration and dried in air (15%). (Found: C, 28.96; H, 5.62; N, 18.60. *Anal.* Calc. for $C_9H_{21}CuN_5O_7$: C, 28.84; H, 5.61; N, 18.69%.) IR (cm^{-1}): 286m, 336vw, 381vw(br), 440w, 548vw, 567vw, 603m, 665w(br), 708m(br), 863m, 927w, 977m, 1011m, 1044m(br), 1086m, 1133m, 1200m, 1215m, 1239m, 1294m, 1307w, 1342m, 1382s, 1433m, 1466w, 1507s(br), 1591s(br), 1673s, 1712m and 2884w. UV–Vis (water): λ633 (ε 90) and 233 nm (1.0×10^4 M^{-1} cm^{-1}). Λ_M/Ω^{-1} cm^2 mol^{-1} (10^{-3} M in water, 20 °C) = 12.0.

2.3. Crystallographic data collection and structure analysis

X-ray data from compounds **1**, **2**, **4** and **5** were collected on an Enraf Nonius CAD4 diffractometer with Mo Kα radiation ($\lambda = 0.71069$ Å). Unit cell parameters were determined from a least squares refinement against 25 reflections randomly searched. Data were collected using the ω–2θ technique. Lorentz-polarization correction and ψ-scan absorption correction were applied using the MolEN [34] package. The structures were solved by direct methods and refined by least squares using SHELXS86 [35] and SHELXL93 [36] for **1** and **4** and SHELX97 [37] for **2** and **5**. The non-hydrogen atoms were anisotropically refined. The hydrogen atoms in the complexes were placed at calculated positions, except those corresponding to the water molecules in **2** and **5**, and those of the NH_2 group in **4**, which were located in difference Fourier maps and restrained to a target value. The hydrogen atoms corresponding to water molecules in **1** could not be located. All the H-atoms were isotropically refined with a global temperature factor in **1** and **4**, and constrained to 1.2 times the Ueq of their bonded atom in **2** and **5** (1.3 times in the case of methyl groups). Details of the data collection and processing are summarized in Table 1.

2.4. Bioactivity assays

Catalase activity was measured by the spectrophotometric method of Aebi [38] based on the decomposition of H_2O_2. SOD-like activity was measured at 37 °C by an adaptation of the McCord and Fridovich method [39]. The xanthine/xanthine oxidase system was used to generate superoxide anion in a reaction mixture containing

Table 1
Crystal data and structure refinement for complexes **1**, **2**, **4** and **5**[a]

	1	**2**	**4**	**5**
	[Cu(gly-L-tyr) Hbim · H_2O	[Cu(L-ala-gly) Hbim] · $3H_2O$	[Cu(gly-L-trp)creat] · $1.25H_2O$	[Cu(L-ala-gly)(H_2O)creat] · $2H_2O$
Empirical formula	$C_{18}H_{20}CuN_4O_5$	$C_{12}H_{20}CuN_4O_6$	$C_{17}H_{20}CuN_6O_4 \cdot 1.25H_2O$	$C_9H_{21}CuN_5O_7$
Formula weight	435.92	379.86	457.45	374.85
Crystal system	orthorhombic	orthorhombic	orthorhombic	triclinic
Space group	$C222_1$	$P2_12_12_1$	$C222_1$	$P1$
a (Å)	9.8360(10)	6.910(3)	14.265(2)	7.299(3)
b (Å)	19.4990(10)	9.867(3)	15.945(2)	7.513(4)
c (Å)	19.5470(10)	23.056(2)	16.920(2)	15.784(4)
α (°)	90	90	90	85.26.(2)
β (°)	90	90	90	86.95(2)
γ (°)	90	90	90	62.93(4)
U (Å^3)	3749.0(5)	1572.0(8)	3848.5(9)	768.0(6)
Z	8	4	8	2
μ(Mo Kα) (cm^{-1})	12.04	14.26	11.8	14.64
Number of reflections measured	2620	4894	2052	4144
Number of unique reflections (R_{int})	2620	4571(0.0397)	1895(0.0335)	4144
Final R_1, wR_2 [$I > 2s(I)$] (all data)	0.0574, 0.0932	0.0506, 0.1184	0.0591, 0.1380	0.0631, 0.1430
	0.2992, 0.1206	0.0937, 0.1295	0.1414, 0.1578	0.2758, 0.1779
Largest difference peak hole (e Å^{-3})	0.554, −1.107	0.462, −0.883	0.600, −0.425	0.893, −1.084

Hbim, benzimidazole; creat, creatinine.
[a] Details in common: $T = 293(2)$ K.

50 mM potassium phosphate buffer, without EDTA at pH 7.8. This anion reduces cytochrome *c*, which was monitored at 550 nm. The Cu(II) complex removed the anion superoxide and produced an inhibition of the reduction which was used as a measure of the enzyme activity. The IC_{50} values were determined by interpolation of the % inhibition versus log of assay concentrations curve for no less than eight points for each system (inhibition values within 5–95% range).

3. Results and discussion

3.1. Description of structures

3.1.1. General features

Bond distances and angles within the benzimidazole or creatinine molecules observed in the corresponding complexes **1**–**3** and **4**–**6** (see Figs. 1–4) are comparable to those obtained earlier for the free benzimidazole [40,41] and creatinine [42] ligands. On the other hand, the peptide dianion has approximately the same chelating angles as observed in other peptide–copper ternary complexes.

3.1.2. [Cu(gly-L-tyr)(benzimidazole)] · H_2O (**1**)

1 Exists as a slightly distorted square planar complex, with the four co-ordination sites occupied by the tridentate glycyl-L-tyrosine dianion [Cu–N(2) = 2.019(8) Å, Cu–N(3) = 1.869(6) Å and Cu–O(1) = 1.980(5) Å] and the N(1) of the benzimidazole moiety [Cu–N(1) = 1.931(8) Å] (Fig. 1). This distance is slightly lower than

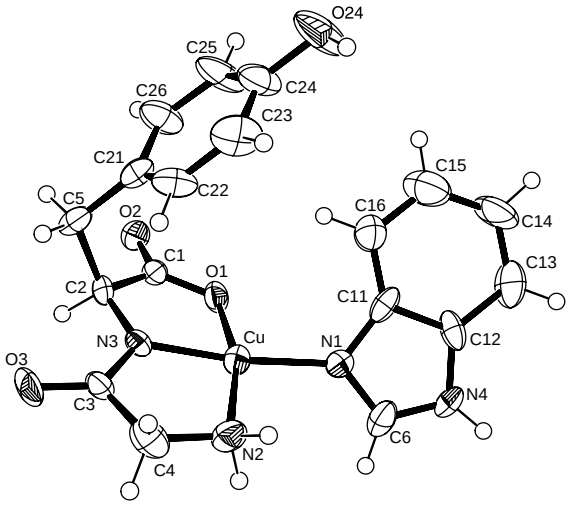

Fig. 1. ORTEP of [Cu(gly-L-tyr)(benzimidazole)] · H_2O (**1**). (Water molecule is omitted for clarity.)

the corresponding distance in the previously described [Cu(gly-gly)(imidazole)] [15,27] [Cu–[N(1)–imidazole] = 1.95 and 1.96 Å] but of the same order to that found in {[Cu(gly-gly)]$_2$(imidazolate)} [Cu–[N(1)-imidazolate] = 1.93 and 1.94 Å] [16]. The values are greater than the previously described [Cu(gly-gly)(benzimidazole)] · $3H_2O$ (**3**) [4] [torsion angles O(1)–Cu–N(1)–C(11) = 56.69° for **1**, 24.89° for **3** and N(2)–Cu–N(1)–C(6) = 50.54° for **1**, 12.91° for **3**]. An interesting non co-planar arrangement of the benzimidazole ligand and the peptide moiety is observed. This very important distortion between the dipeptide plane and the benz-

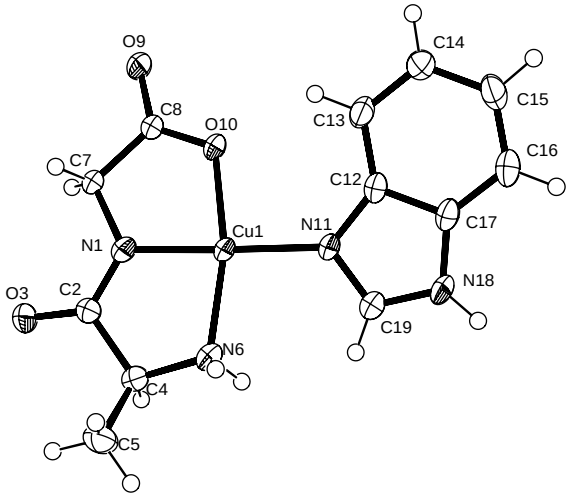

Fig. 2. ORTEP of [Cu(L-ala-gly)(benzimidazole)] · $3H_2O$ (**2**). (Water molecules are omitted for clarity.)

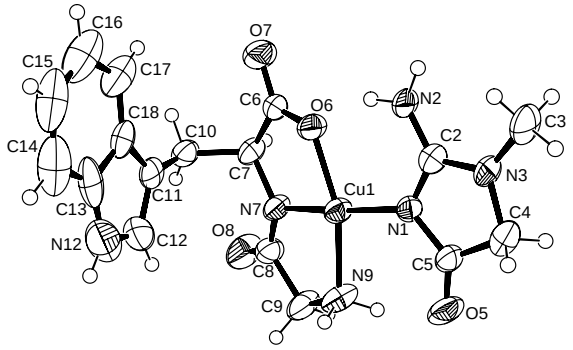

Fig. 3. ORTEP of [Cu(gly-L-trp)(creatinine)] · $1.25H_2O$ (**4**). (Water molecules are omitted for clarity.)

imidazole ring produces the elimination of C–H···O hydrogen bond interaction between a C–H of the benzimidazole moiety and the oxygen of the carboxylic group of the peptidic moiety that exists in **3** and **2** (see below) but favours a weak intramolecular CH···π interaction [43,44] {C(16)–H···tyrosine aromatic ring [C(23), C(24), C(25)] = 3.20 ± 0.05 Å}. The crystal packing is dominated by two relatively strong hydrogen bonds between monomeric units, the phenolic oxygen O(24) with the peptide carboxylic oxygen O(2) [O(24)–H···O(2) = 1.874 Å] and the N(4) of benzimidazole with the peptide carboxamide oxygen O(3) [N(4)–H···O(3) = 1.909 Å]. Other intermolecular hydrogen contacts can be appreciated between the peptide amino group N(2) with the phenolic oxygen O(24), peptide carboxylic oxygen O(2) and a water molecule O(4) [distances: N(2)–H···O(24) = 2.22 Å, N(2)–H···O(2) = 2.30 Å and N(2)–H···O(4) = 2.46 Å].

3.1.3. [Cu(L-ala-gly)(benzimidazole)] · $3H_2O$ (**2**)

The molecular structure of [Cu(L-ala-gly)(benzimidazole)] · $3H_2O$ (**2**) is shown in Fig. 2. The Cu(II) ion has approximately square planar co-ordination, being linked to the chelating atoms N(6) α-amino nitrogen [Cu–N(6) = 2.005(3) Å], N(1) amide nitrogen [Cu–N(1) = 1.896(3) Å] and O(10) carboxyl oxygen [Cu–O(10) = 1.959(3)] of the L-alanylglycine dianion and N(11) of the benzimidazole molecule [Cu–N(11) = 1.959(3) Å] (Table 2). This distance is identical to the corresponding distance for the previously described [Cu(gly-gly)(imidazole)] [15] [Cu–[N(1)–imidazole] = 1.95 and 1.96 Å]. [Cu(gly-gly)(benzimidazole)] · $3H_2O$ (**3**) [4] shows an identical value of Cu–N(1) [1.962(4) Å] and a greater Cu–N(6) [2.029 Å] distance than complex **2**. Moreover, a similar nearly coplanar arrangement of the benzimidazole ligand and the peptide moiety is observed [torsion angles O(10)–Cu–N(11)–C(12) = 20.80° for **2**, 24.89° for **3** and N(6)–Cu–N(11)–C(19) = 16.52° for **2**, 12.91° for **3**]. This slight distortion between the dipeptide plane and the benzimidazole ring, in **2** and **3**, favours the interesting intramolecular CH···O hydrogen bond interaction [45–48] previously described for **3** [C(13)–H···O(10) = 2.29 Å

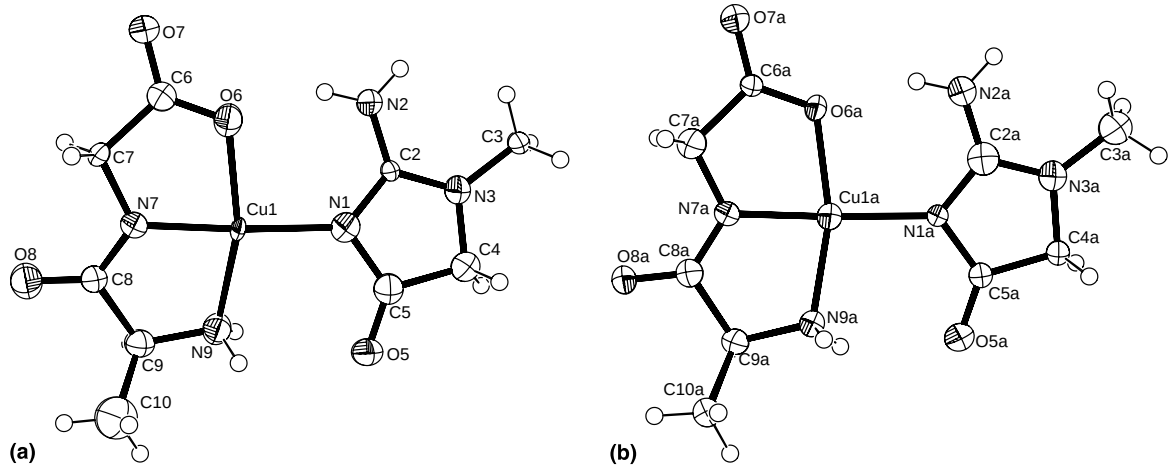

Fig. 4. (a, b) ORTEP of [Cu(L-ala-gly)(H_2O)(creatinine)] · $2H_2O$ (**5**) (two different complex units are present in the asymmetric unit). (Water molecules are omitted for clarity.)

Table 2
Selected bond lengths (Å) and angles (°) for complexes **1**, **2**, **4** and **5**

[Cu(gly-L-tyr)(benzimidazole)]·H_2O (**1**)			
Bond lengths			
Cu–N(3)	1.869(6)	Cu–N(1)	1.931(8)
Cu–O(1)	1.980(5)	Cu–N(2)	2.019(8)
Bond angles			
N(3)–Cu–N(1)	171.2(3)	N(3)–Cu–N(2)	82.9(3)
N(3)–Cu–O(1)	83.1(3)	N(1)–Cu–N(2)	98.6(3)
N(1)–Cu–O(1)	96.4(3)	O(1)–Cu–N(2)	164.2(3)
[Cu(L-ala-gly)(benzimidazole)]·3H_2O (**2**)			
Bond lengths			
Cu–N(1)	1.896(3)	Cu–N(11)	1.959(3)
Cu–O(10)	1.959(3)	Cu–N(6)	2.005(3)
Bond angles			
N(1)–Cu–O(10)	83.30(13)	N(1)–Cu–N(6)	83.10(14)
N(1)–Cu–N(11)	177.5(2)	O(10)–Cu–N(6)	166.39(13)
O(10)–Cu–N(11)	95.79(13)	N(11)–Cu–N(6)	97.82(14)
[Cu(gly-L-trp)(creatinine)]·1.25H_2O (**4**)			
Bond lengths			
Cu-N-(7)	1.892(9)	Cu–N(1)	1.996(9)
Cu–O(6)	1.955(7)	Cu–N(9)	2.011(8)
Bond angles			
N(7)–Cu–O(6)	81.1(3)	N(7)–Cu–N(9)	83.4(4)
N(7)–Cu–N(1)	176.5(4)	O(6)–Cu–N(9)	162.6(4)
O(6)–Cu–N(1)	96.7(3)	N(1)–Cu–N(9)	99.1(4)
[Cu(L-ala-gly)(H_2O)(creatinine)]·2H_2O (**5**)			
Bond lengths			
Cu(1)–N(7)	1.900(9)	Cu(1A)–N(7A)	1.897(10)
Cu(1)–O(6)	2.028(9)	Cu(1A)–O(6A)	2.027(9)
Cu(1)–N(1)	2.011(9)	Cu(1A)–N(1A)	2.005(9)
Cu(1)–N(9)	2.013(10)	Cu(1A)–N(9A)	2.011(9)
Cu(1)–O(11)	2.40(2)	Cu(1A)–O(14)	2.45(2)
Cu(1)–O(12)	2.83(2)	Cu(1A)–O(15)	3.01(2)
Bond angles			
N(7)–Cu(1)–N(1)	174.6(8)	N(7A)–Cu(1A)–N(1A)	174.4(8)
N(7)–Cu(1)–N(9)	82.6(5)	N(7A)–Cu(1A)–N(9A)	81.8(4)
N(1)–Cu(1)–N(9)	98.8(5)	N(1A)–Cu(1A)–N(9A)	99.6(5)
N(7)–Cu(1)–O(6)	81.4(5)	N(7A)–Cu(1A)–O(6A)	81.1(4)
N(1)–Cu(1)–O(6)	96.6(5)	N(1A)–Cu(1A)–O(6A)	96.8(5)
N(9)–Cu(1)–O(6)	162.8(5)	N(9A)–Cu(1A)–O(6A)	162.0(5)
N(7)–Cu(1)–O(11)	96.5(7)	N(7A)–Cu(1A)–O(14)	98.0(7)
N(1)–Cu(1)–O(11)	88.7(7)	N(1A)–Cu(1A)–O(14)	87.4(6)
N(9)–Cu(1)–O(11)	91.9(7)	N(9A)–Cu(1A)–O(14)	89.6(6)
O(6)–Cu(1)–O(11)	96.2(7)	O(6A)–Cu(1A)–O(14)	98.6(6)
N(7)–Cu(1)–O(12)	89.0(7)	N(7A)–Cu(1A)–O(15)	89.4(7)
N(1)–Cu(1)–O(12)	85.8(7)	N(1A)–Cu(1A)–O(15)	85.0(6)
N(9)–Cu(1)–O(12)	89.4(7)	N(9A)–Cu(1A)–O(15)	94.1(6)
O(6)–Cu(1)–O(12)	84.0(6)	O(6A)–Cu(1A)–O(15)	79.9(6)
O(11)–Cu(1)–O(12)	174.5(7)	O(14)–Cu(1A)–O(15)	172.1(5)

for **2** and 2.31 Å for **3**]. Like in complex **3**, the monomeric units are linked by two different types of hydrogen bonds: (a) a normal hydrogen contact N(18)–H···O(9) [distance N(18)–H···O(9) = 1.85 Å]; (b) a large interaction which implies an adjacent water molecule O(3)···H–(OW)···H–N(6) [distance O(3)···N(6) = 4.60 Å]. The crystal packing involves a tridimensional network of water molecules.

3.1.4. *[Cu(gly-L-trp)(creatinine)]·1.25H_2O* (**4**)

4 Exists as a slightly distorted square planar complex, with the four co-ordination sites occupied by the tridentate glycyl-L-tryptophanato dianion [Cu–N(9) = 2.011(8) Å, Cu–N(7) = 1.892(9) Å and Cu–O(6) = 1.955(7) Å] and the N(1) of the creatinine moiety [Cu–N(1) = 1.996(9) Å] (Fig. 3). These values are similar to the previously described [Cu(gly-gly)(H_2O)(creati-

nine)]·1.5H_2O (**6**) [5]. A co-planarity between the peptide and creatinine moieties is observed again, which can be explained by two intramolecular hydrogen bonds [N(2)–H···O(6) = 1.83 Å and N(9)–H···O(5) = 2.25 Å]. The torsion angles are lower than the previously described [Cu(glygly)(H_2O)(creatinine)]·1.5H_2O (**6**) complex [5] [torsion angles O(6)–Cu–N(1)–C(2) = −3.38° for **4**, −8.95° for **6** and N(9)–Cu–N(1)–C(5) = 7.37° for **4**, −19.35° for **6**]. The crystal packing is dominated by two relatively strong hydrogen bonds between monomeric units, the amine group of the creatinine ligand N(2) with the peptide carboxamide oxygen O(8) [N(2)–H···O(8) = 1.95 Å] and the amine group of the peptide N(9) with the creatinine carboxamide oxygen O(5) [N(9)–H···O(5) = 2.24 Å]. Other intermolecular hydrogen contacts can be appreciated between the indolic nitrogen of the lateral chain of the peptide moiety N(12) and different water molecules [distances: N(12)–H···O(W) = 1.97 and 2.22 Å].

3.1.5. [Cu(L-ala-gly)(H_2O)(creatinine)]·2H_2O (**5**)

The molecular structure of [Cu(L-ala-gly)(H_2O)(creatinine)]·2H_2O (**5**) is shown in Fig. 4(a) and (b) and presents an analogous structure to the previously described [Cu(gly-gly)(H_2O)(creatinine)]·1.5H_2O (**6**) [5]. Two similar molecular units are presented in the crystalline asymmetrical unit where the Cu(II) ion has approximately square-pyramidal co-ordination being linked to the chelating atoms N(9) α-amino nitrogen [Cu–N(9) or N(9A) = 2.013(10) and 2.011(9) Å], N(7) amide nitrogen [Cu–N(7) or N(7A) = 1.900(9) and 1.897(9) Å] and O(6) carboxyl oxygen [Cu–O(6) or O(6A) = 2.028(9) and 2.027(8) Å] of the L-alanyl-glycine dianion and N(1) of the creatinine molecule [Cu–N(1) or N(1A) = 2.011(9) and 2.005(9) Å] (Table 2). This distance is higher than that corresponding to the previously described [Cu(gly-gly)(imidazole)] [15] [Cu–[N(1)–imidazole] = 1.95 and 1.96 Å] and similar to other Cu–imidazole complexes [49]. A water molecule completes the co-ordination sphere about the copper atom [Cu–O(11) or O(14) = 2.40(2) and 2.45(2) Å]. As mentioned in complex **4**, nearly co-planar arrangements of the creatinine ligand and the peptide moiety are present in L-ala-gly (**5**) and gly-gly (**6**) complexes, which are produced by a tandem of intramolecular hydrogen bonds [N(2)–H···O(6) = 1.99 and 1.95 Å (**5**), 1.97 Å (**6**) and N(9)–H···O(5) = 2.38 and 2.24 A (**5**), 2.32 (**6**)]. Values of torsion angles are: O(6)–Cu–N(1)–C(2) = −9.96° and 9.78° for **5**, −8.95° for **6** and N(9)–Cu–N(1)–C(5) = −25.70° and 12.58° for **5**, −19.35° for **6**. The crystal packing involves, like in complex **3** [5], a tridimensional network of water molecules. Only additional H bonds that imply interaction between the two units of the crystallographic asymmetrical unit [N(2)H···O(7A) = 2.07 and N(2A)H···O(7) = 2.13 Å] should be indicated.

3.1.6. General discussion of complex structures

A comparison of the binding modes of the [Cu(II)–dipeptide system and the heterocyclic ligands in **1**–**6** reveals a general tendency of a nearly co-planar arrangement (**2**–**6**) between the ligand and dipeptide planes which is stabilized by additional weak interactions. Only in [Cu(gly-L-tyr)(benzimidazole)]·H_2O (**1**), is a lack of co-planarity present. In this case, the presence of a benzene ring in the lateral chain could determine this non-co-planar arrangement, which implies the substitution of a specific weak CH···O additional intramolecular hydrogen bond interaction (present in **2** and **3**) for a weak CH···π interaction between the peptide–Cu(II) system and benzimidazole. Moreover, the Cu–N(benzimidazole) bond lengths are of the same order to the corresponding Cu–N(imidazole) complexes according to the expected back bonding from Cu(II) *to* the empty π^* orbitals of the aromatic system.

In the case of creatinine, the presence of two important intramolecular hydrogen bonds involving the exocyclic NH_2 and C=O groups of the creatinine molecule and the corresponding groups in the peptide moiety yields square planar compounds, independent of the nature of the lateral chain present in the peptidic group [O(6)···HN(2) (1.83 for **4**, 1.99 and 1.95 for **5** and 1.97 Å for **6**) and N(9)H···O(5) (2.25 for **4**, 2.38 and 2.24 for **5** and 2.32 Å for **6**)]. This behavior seems to be characteristic of creatinine because the identical relative position of these groups (NH_2 and C=O) in other ligands such as cytosine, cytidine, isocytosine or methylisocytosine, does not imply necessarily the double formation of H-bonds [NH_2(ligand)···O–C(O) (peptide) and C=O(ligand)···NH_2(peptide)]. In these latter compounds only a weaker C=O(ligand)···NH_2 (peptide) H-bond [3.28 Å for isocytosine [8,21], 3.22 Å for methylisocytosine [8], 3.30 Å for cytidine [9] and 3.97 Å for cytosine [10] versus 2.78 to 2.82 for creatinine complexes] and a great deviation from planarity between the peptide and the ligand are observed.

3.2. IR spectra

3.2.1. Benzimidazole complexes **1**–**3**

The IR spectra of **1** and **2** have been compared with the corresponding IR spectra of the [benzimidazole ligand [2]

[2] Tentative band assignments (cm^{-1}) for benzimidazole according to the literature [42,50] are: [ν(C=C) skeletal in-plane vibrations of benzene, ν(ring), ν(C–C) + ν(C–N)] = 1623w,1604vw; [δNH(in-plane bending) + ν(CN)] = 1591m; [ν(ring), δ(CH) + ν(CC)] = 1480m; [ν(CN) + δNH] = 1461s; [νCN + δ(ring)(Im)] = 1412vs; [ν(CC) + ν(CN)] = 1367m; [in plane CH deformations and ring-breathing modes] = 1250–1000; [δ(CH) + ν(ring)(Im)] = 1248s; [ν(CN) + δNH (NH in-plane bending)] = 1137m; [δ(ring) + δCH(Im)] = 1006m; [ν(CC) + (δ(NCN)) ring (Im)] = 960m; [heterocyclic ring breathing modes and out of plane CH bending frequencies] = 888m, 770s, 747s; [ring torsion γCH(benz)] = 423m.

[50] and ternary [Cu(gly-gly) (benzimidazole)] · $3H_2O$ (**3**) [4] previously reported. Thus, a very strong and broad band is observed [1612 cm^{-1} for **1** and 1585 cm^{-1} for **2**] which is assignable to ν(C=O) + ν(C–N), and ν_a(COO) of the peptidic moiety [51] with contribution of the δN–H + ν(C–N) and ν(C=C) skeletal in plane vibrations of benzene peaks of the benzimidazole ligand [50]. The strong bands at 1480 and 1461 cm^{-1} which have contribution of ν(ring), ν(C–C) and ν(C–N) in the free benzimidazole ligand spectrum are resolved into the weak band at 1473 cm^{-1} upon complexation, and the very strong 1412 cm^{-1} band corresponding to ν(CN) + δ(ring)(Im) in the free ligand [2] is missing in the spectra of the complexes. These changes could be diagnostic for co-ordinated benzimidazole Cu(II)-complexes. Moreover, in the case of [Cu(gly-L-tyr)benzimidazole] · H_2O (**1**) a broad absorption band centred around 3200 cm^{-1} is observed, which is attributed to the presence of co-ordinated water ν(O–H), from which emerges the sharp peak at 3640 cm^{-1} assignable to free ν(OH). The bands appearing at 366, 325 and 299 cm^{-1} for **1** and 349, 321 and 310 cm^{-1} for **2** are related to ν(Cu–O) and ν(Cu–N) [52].

3.2.2. Creatinine complexes **4**–**6**

The infrared spectra of the two complexes were compared with that of the creatinine ligand and ternary [Cu(gly-gly)(H_2O)(creatinine)] · $2H_2O$ (**6**) [5]. Tentative assignments for several complexation-sensitive bands of creatinine have been proposed [25,53]. In the infrared spectra of **4** and **5**, the bands corresponding to the $\nu_{as}(NH_2)$ and $\nu_s(NH_2)$ vibrations (two peaks at 3343m, 3285m emerging from a broad band for **4** and a very broad band centred ca. 3250s cm^{-1} for **5**) confirm the presence of creatinine in its amino tautomer form with the NH_2 group probably involved in hydrogen bonds. Co-ordination of creatinine to Cu(II) through N(1) results in an increase in the frequency of the ν(C(5)=O) [1692 cm^{-1} for creatinine] in both cases [1725 for **4** and 1712 cm^{-1} for **5**] as observed in other structurally known N(1)-metallated complexes. These data suggest co-ordination of Cu(II) to the heterocyclic nitrogen of creatinine in both compounds.

3.3. Electronic spectra

The compounds yield blue solutions in methanol **1** and water **2**, **4** and **5**. The d–d transition spectra of the complexes present broad bands centred at 616 for **1**, 624 for **2**, 628 for **4** and 633 nm for **5** and are suggestive of approximately square pyramidal geometry about Cu(II) as observed in other Cu(II) peptide complexes. The π–π^* transition band (243 nm) involving the imidazole moiety of the benzimidazole ligand is overlapped in the spectrum of complex **1** in methanol with the stronger broad band at 225 nm corresponding to the benzene ring of the tyrosyl moiety, and exhibits little change in intensity appearing at 241 nm for **2** in water. The π–π^* intraligand transitions involving the benzene ring (271 and 278 nm) are rather insensitive to metal complex formation appearing at 272 and 279 nm for **1** and 271 and 278 nm for **2**. On the other hand, the π–π^* transition band of creatinine in water solution (234 nm) increases its relative intensity in the spectrum of complex **5** appearing at 233 nm whereas in the spectrum of complex **4** the π–π^* transition bands of the indole moiety (221 and 281 nm) overlap completely with the creatinine π–π^* transition band. The Λ_M (10^{-3} mol dm^{-3}) values in methanol (for **1**) and water (for **2**, **4** and **5**) at 20 °C (16.4, 6.3, 16.1 and 12.0 Ω^{-1} cm^2 mol^{-1}, respectively) imply the presence of non-electrolyte species [54].

3.4. ESR spectra and magnetic properties

Magnetic measurements show that there is no coupling between the unpaired electrons on the copper(II) centres. The effective magnetic moments μ_{eff} of 1.82 BM (for **1**), 1.77 BM (for **2**), 1.94 BM (for **3**), 1.84 BM (for **4**), 1.76 BM (for **5**) and 1.82 BM (for **6**) at room temperature are normal for magnetically diluted d^9 systems. The X-band ESR spectra of polycrystalline samples of the complexes recorded at room temperature do not show any hyperfine splittings. These spectra are of the axial type with the values: $g_\parallel = 2.18$ and $g_\perp = 2.09$ (for **1**); $g_\parallel = 2.19$ and $g_\perp = 2.05$ (for **2**–**4**); $g_\parallel = 2.21$ and $g_\perp = 2.07$ (for **5**) and $g_\parallel = 2.21$ and $g_\perp = 2.06$ (for **6**). They agree with slightly distorted square planar or square-pyramidal Cu(II) complexes. Simulated spectra obtained by using the Brucker WINEPR program gave good agreement with the experimental g values.

3.5. Bioactivity Studies

The complexes neither catalyze the dismutation of hydrogen peroxide (lack of catalase-like activity), showing the typical behavior of free copper(II), nor present a remarkable SOD-like activity. On the other hand, although IC_{50} values are similar or higher than free Cu(II) it is possible to define two different series of ternary metal complexes: Benzimidazole complexes with similar values to $CuSO_4 \cdot 5H_2O$ {IC_{50} values: $CuSO_4 \cdot 5H_2O$ = 65.7±3.5; Cu(gly-L-tyr)(benzimidazole)]·H_2O (**1**)=61.3± 4.0; [Cu(L-ala-gly)(benzimidazole)] · $3H_2O$ (**2**)=49.9±3.1 [Cu(gly-gly)(benzimidazole)] · $3H_2O$ (**3**) = 61.9 ± 2.9} and creatinine derivatives with higher values than $CuSO_4 \cdot 5H_2O$ {IC_{50} values: $CuSO_4 \cdot 5H_2O$ = 65.7 ± 3.5; [Cu(gly-L-trp)(creatinine)] · $1.25H_2O$ (**4**)=166.8±5.3 and [Cu(L-ala-gly)(H_2O)(creatinine)] · $2H_2O$ (**5**) = 82.0 ± 3.3} [55]. The SOD-like activity seems to be related to a change or distortion of the geometry around the metal centre and steric hindrance related to the approach of the

O_2^- anion as well as fast interchange of axial co-ordinated solvent molecules [31,32,56]. Thus, although we cannot discard a labilization or break of the two hydrogen bonds in solution, a possible explanation for the lack of activity in co-planar creatinine complexes, comparing to free Cu(II), could be related to the less flexibility of these Cu(II) systems due to the presence of a tandem of hydrogen bonds that held creatinine in a nearly co-planar disposition with the peptide moiety. A certain structural mobility, necessary for the stabilization of Cu(I) generated by the one-electron reduction by the superoxide anion, should be necessary. Moreover, [Cu(gly-L-trp)(creatinine)] · $1.25H_2O$ (**4**), the less active complex, could be related to the "extra" steric hindrance produced by the indole moiety of the peptide lateral chain that makes the approach of the O_2^- anion to the Cu centre more difficult.

4. Conclusion

The interaction between the Cu(II)-dipeptide system and benzimidazole or creatinine reveals a general tendency of nearly co-planar complexes **1**–**6**, which are always stabilized by additional weak interactions that assist to the final co-planar arrangement in **2**–**6**. Thus, the specific hydrogen bond pattern between the two complementary components: peptide moiety and creatinine seems to be independent on the nature of the peptidic lateral chain. Contrarily as in the benzimidazole complexes, it is not possible to establish such a specific hydrogen bond pattern and only weak additional interactions could be present, the relative orientation is highly influenced by the nature of the lateral chain of the peptide and it is possible to obtain non-co-planar complexes when the lateral chain of the peptidic moiety permits a re-orientation of the ligand.

5. Supplementary material

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 183540 for **1**, 183541 for **2**, 183542 for **4** and 183543 for **5**. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www:http://www.ccdc.cam.ac.uk).

Acknowledgements

We are grateful to Spanish CICYT (PB98-0123 and BQU2002-02546) and Vicerectorat d'lnvestigació de la Universitat de les Illes Balears for financial support.

References

[1] H. Sigel (Ed.), Metal Ions in Biological Systems, vol. 12, Marcel Dekker, New York, 1981.
[2] H. Sigel (Ed.), Metal Ions in Biological Systems, vol. 13, Marcel Dekker, New York, 1981.
[3] T. Miura, A. Hori-i, H. Mototani, H. Takeuchi, Biochemistry 38 (1999) 11560.
[4] A. García-Raso, J.J. Fiol, B. Adrover, E. Molins, C. Miravitlles, Polyhedron 15 (1996) 1829.
[5] A. García-Raso, A. Terrón, J.J. Fiol, E. Molins, C. Miravitlles, Polyhedron 14 (1995) 2537.
[6] K. Saito, R. Terashima, T. Sakaki, K. Tomita, Biochem. Biophys. Res. Commun. 61 (1974) 83.
[7] T.J. Kistenmacher, D.J. Szalda, L.G. Marzilli, Acta Crystallogr., Ser. B 31 (1975) 2416.
[8] A. García-Raso, J.J. Fiol, B. Adrover, V. Moreno, E. Molins, I. Mata, J. Chem. Soc., Dalton Trans. (1998) 1031.
[9] D.J. Szalda, L.G. Marzilli, T.J. Kistenmacher, Biochem. Biophys. Res. Commun. 63 (1975) 601.
[10] D.J. Szalda, T.J. Kistenmacher, Acta Crystallogr., Ser. B 33 (1977) 865.
[11] T.J. Kistenmacher, L.G. Marzilli, D.J. Szalda, Acta Crystallogr., Ser. B 32 (1976) 186.
[12] L.G. Marzilli, K. Wilkowski, C.C. Chiang, T.J. Kistenmacher, J. Am. Chem. Soc. 101 (1979) 7504.
[13] M.C. Lim, E. Sinn, R.B. Martin, Inorg. Chem. 15 (1976) 807.
[14] C.J. Simmons, M. Lundeen, K. Seff, Inorg. Chem. 17 (1978) 1429.
[15] J.D. Bell, H.C. Freeman, A.M. Wood, R. Driver, W.R. Walker, Chem. Commun. (1969) 1441.
[16] K. Matsumoto, S. Ooi, Y. Nakao, W. Mori, A. Nakahara, J. Chem. Soc., Dalton Trans. (1981) 2045.
[17] F.S. Wang, A.L. Cui, H.M. Chen, Y.J. Zhao, Gaodeng Xuexiao Huaxue Xuebao (Chin.) (Chem. J. Chim. Uni.) 15 (1994) 319 [Compound NUVBUB in CCDC].
[18] X.L. Guo, W.X. Zhang, A.L. Cui, L.C. Shen, F.S. Wang, Z.L. Li, Gaodeng Xuexiao Huaxue Xuebao (Chin.) (Chem. J. Chim. Uni.) 19 (1998) 629 [Compound MEHYED in CCDC].
[19] T. Sugimori, K. Shibakawa, H. Masuda, A. Odani, O. Yamauchi, Inorg. Chem. 32 (1993) 4951.
[20] A. García-Raso, J.J. Fiol, B. Adrover, V. Moreno, I. Mata, E. Espinosa, E. Molins, J. Inorg. Biochem. 95 (2003) 77.
[21] A. García-Raso, J.J. Fiol, B. Adrover, A. Caubet, E. Espinosa, I. Mata, E. Molins, Polyhedron 21 (2002) 1197.
[22] M. Mitewa, Coord. Chem. Rev. 140 (1995) 1.
[23] A.J. Canty, M. Fyfe, B.M. Gatehouse, Inorg. Chem. 17 (1978) 1467.
[24] J.M. O'Connor, K. Hiibner, A.L. Rheingold, L.M. Liable-Sands, Polyhedron 16 (1997) 2029.
[25] B.S Parajon-Costa, E.J. Baran, O.E. Piro, Polyhedron 16 (1997) 3379.
[26] A. Panfil, J.J. Fiol, M. Sabat, J. Inorg. Biochem. 60 (1995) 109.
[27] R.J. Sundberg, R.B. Martin, Chem. Rev. 74 (1974) 471.
[28] P.N. Preston, Chem. Rev. 74 (1974) 279.
[29] M. Sanchez-Moreno, E. Entrala, D. Janssen, C. Fernandez-Becerra, J.M. Salas-Peregrin, A. Osuna, Pharmacology 52 (1996) 61.
[30] V.L. Zacny, E. Gershburg, M.G. Davis, K.K. Biron, J.S. Pagano, J. Virol. 73 (1999) 7271.
[31] A.L. Abuhijleh, J. Inorg. Biochem. 68 (1997) 167, and references therein.
[32] J. Casanova, G. Alzuet, J. Borrás, J. Latorre, M. Sanaú, S. Garcia-Granda, J. Inorg. Biochem. 60 (1995) 219.
[33] A.R. Manyak, C.B. Murphy, A.E. Martell, Arch. Biochem. Biophys. 59 (1955) 373.

[34] C.K. Fair, MolEn, An Interactive Intelligent System for Crystal Structure Analysis, Enraf-Nonius, Delft, 1990.
[35] G.M. Sheldrick, Acta Crystallogr., Sect. A 46 (1990) 467.
[36] G.M. Sheldrick, SHELXL93. Program for crystal structure refinement, University of Cambridge, Cambridge, 1993.
[37] G.M. Sheldrick, SHELXL97. Programs for crystal structure analysis, Institut fur Anorganische Chemie der Universität, Göttingen, 1998.
[38] H.E. Aebi, in: H.U. Bergmeyer (Ed.), HE Methods in Enzymatic Analysis, Verlag Chemie, Basel, 1984, p. 273–286.
[39] J. McCord, I. Fridovich, J. Biol. Chem. 244 (1969) 6049.
[40] C.J. Dik-Edixhoven, H. Schenk, H. van der Meer, Cryst. Struct. Commun. 2 (1973) 23.
[41] A. Quick, D.J. Williams, Can. J. Chem. 54 (1976) 2482.
[42] S. Du Pré, H. Mendel, Acta Crystallogr. 8 (1955) 311.
[43] H.C. Weiss, D. Bläser, R. Boese, B.M. Doughan, M.M. Haley, Chem. Commun. (1997) 1703.
[44] N.N. Laxmi Madhavi, A.M. Katz, H.L. Carrell, A. Nangia, G.R. Desiraju, Chem. Commun. (1997) 1953.
[45] T. Steiner, Chem. Commun. (1997) 727.
[46] S.G. Bodige, R.D. Rogers, S.C. Blackstock, Chem. Commun. (1997) 1669.
[47] V.R. Thalladi, S. Brasselet, D. Bläser, R. Boes, J. Zyss, A. Nangia, G.R. Desiraju, Chem. Commun. (1997) 1841.
[48] F.A. Cotton, L.M. Daniels, G.T. Jordan IV, C.A. Murillo, Chem. Commun. (1997) 1673.
[49] F. Akhtar, D.M.L. Goodgame, M. Goodgame, G.W. Rayner-Canham, A.C. Skapski, Chem. Commun. (1968) 1389.
[50] M.M. Cordes, J.L. Walter, Spectrochim. Acta 24A (1968) 1421.
[51] K. Nakamoto, Infrared and Raman spectra of inorganic and coordination compounds, Wiley, New York, 1978, p. 243.
[52] G.A. Melson, R.H. Nuttall, J. Mol. Struct. (1967-68) 405.
[53] N. Trendafilova, A.P. Kurbakova, I.A. Efimenko, M. Mitewa, P.R. Bontchev, Spectrochim. Acta 47A (1991) 577.
[54] W.J. Geary, Coord. Chem. Rev. 7 (1971) 81.
[55] The limitations and controls that are necessary to validate and assess the SOD activity assay have been taken into account: D.P. Riley, Chem. Rev. 99 (1999) 2573.
[56] S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, CA, 1994, pp. 325–329.