Research paper

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Development of novel Cu(I) compounds with vitamin B_1 derivative and their potential application as anticancer drugs

Paula Brandão[a]*, Samuel Guieu[a,b], Ana Correia-Branco[c], Cláudia Silva[c], Fátima Martel[c]*

[a]Departamento de Química, CICECO, Universidade de Aveiro, 3810-193 Aveiro, Portugal.
[b] Departamento de Química,QOPNA, Universidade de Aveiro, 3810-193 Aveiro, Portugal.
[c]Departamento de Biomedicina, Unidade de Bioquímica, Faculdade de Medicina do Porto e I3S, Universidade do Porto, 4200-319, Porto, Portugal

* Corresponding authors e-mail address: pbrandao@ua.pt; fmartel@med.up.pt

Abstract

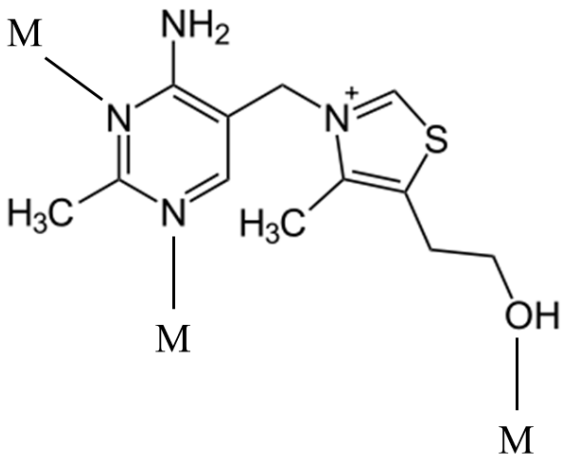
The synthesis and crystal structure of two new copper(I) compounds with molecular formula of $\{Cu_4[(\mu_3\text{-thiocrome})_2Cl_4]\cdot 2(H_2O)\}_n$ **1**, and $Cu_2[(\mu_2\text{-thiocrome})_2Cl_2]$ **2** are reported. The crystal structure of compounds **1** and **2** are solved by single crystal X-ray diffraction. The reaction of Cu(II) with thiamine chloride in water at room temperature produces Cu(I) thiochrome compounds **1** and **2**. Compound **1** shows a 1D chain structure based on the linkage of two crystallographic different copper centers and thiochrome ligand through the N(1), N(2) and N(3) nitrogen atoms. Compound **2** is a 0D dimeric copper structure assembled by two thiochrome ligands. For both compounds, the copper(I) centers exhibits a distorted trigonal pyramidal geometry. The antitumor capacity of both compounds was tested *in vitro* against a human cancer cell line, the colorectal adenocarcinoma (Caco-2) cell line, by determining their effect on cell viability. The two compounds showed similar IC_{50} values, and were slightly more potent than cisplatin, against the same cell line.

Keywords: Copper(I) , vitamin B_1 derivatives, thiochrome, Caco-2 cells.

1. Introduction

Over the past decades, metal complexes have had enormous success as cytotoxic drugs against cancer related diseases. Cisplatin, cis-$[Pt(NH_3)_2Cl_2]$, is the quintessential example of a "small" transition metal complex which had been widely used as an anti-tumoral drug, particularly against testicular, ovarian, bladder, and head/neck tumours [1-3]. However, its effectiveness was early clouded by several undesirable side effects along with acquired drug resistance. This clearly showed that selectivity and specificity are indeed the main keys to design and produce better anti-tumoral new drugs. Because of these drawbacks, the development of other anticancer metal-based drugs with more specificity and less toxicity is nowadays the

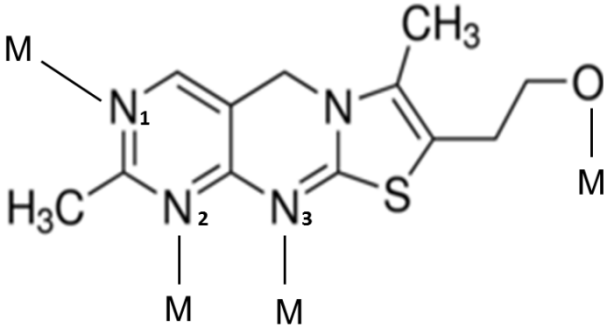
object of the attention of a great number of researchers, who have directed their efforts towards the synthesis of Ti, Au, Pd, Ru, Rh, Ir, Mo, and Cu [4-15] complexes which can overcome both the natural and the acquired resistance of human cancer cells to cisplatin and analogous drugs. Among this array of metal complexes, those with Cu have been much less explored, which seems to be a paradox taking into account the availability and bio-compatibility of copper. A further benefit of the use of this metal is that it is much cheaper than the "traditional" metals (platinum, ruthenium, rhodium and gold) currently used in the preparation of metal-based anticancer agents. A considerable number of Cu(II) complexes have been reported as potential anti-tumour agents and they have been found to be active both *in vitro* and *in vivo* [8-14]. Under physiological conditions Cu(II) species could be reduced to more toxic Cu(I). To overcome the in vivo reduction the use of Cu(I) complexes might be a positive option. In literature we can find a few works with Cu(I) complexes and their investigation as potential anticancer agents [16-20]. Our group in Aveiro has explored the design and synthesis of novel Cu complexes with potential anticancer properties using natural ligands such as adenine, a DNA base, and vitamin B_3, producing compounds more selective, less toxic and with better therapeutic indices than today's drugs [21, 22]. As an extension of this work we chose vitamin B_1 (also known as thiamine) as the organic ligand to prepare novel Cu complexes. Vitamin B_1 is composed of an aminopyrimidine and a thiazole ring linked by a methylene bridge. This biomolecule exhibits monodentate, bidentate and bridging metal coordination modes due to the presence of the aminopyrimidine ring and hydroxyl group (scheme 1). In literature, complexes of several metals (Hg, Cd, Zn, Pt, Cu, Co, Mn and Rh) with vitamin B_1 or derivatives like as oxythiamine, 2-(α-hydroxybenzyl) thiamine, thiamine monophosphate and thiamine pyrophosphate have been described [23-40]. All these complexes show the metal coordinated to the N(1) atom from the aminopyrimidine ring in a monodentate mode and the metal coordination sphere is completed by water molecules, chloride or bromide ions as well as thiocyanato ions. Two other complexes with Mn and Cd are known in which the metal is coordinated to N(1) and also to the oxygen atom from the hydroxyethyl group, forming di-nuclear metal complexes [41, 42]. Aoki and co-workers synthesised a cadmium thiamine polymeric structure in which the octahedral Cd(II) bond thiamine ligand through the hydroxyethyl oxygen [43].



Scheme 1 Different coordination modes of vitamin B_1 (M= metal)

Continuing our research on the interaction of Cu ions with vitamin B_1 at room temperature we realised that thiamine is oxidised to thiochrome and the copper centre coordinated to this ligand moiety *via* N(1) and N(2) atoms from the pyrimidine ring, and N(3) atom from hexamine ring (scheme 2). The oxidation of thiamine in the presence of Cu(II) species has already been described in literature [44, 45]. Louloudi and co-workers in 1997 prepared one polymeric structure of $\{Cu^{II}(thiochrome)Cl_2\}_n$ by the reaction of $CuCl_2$ with thiamine in a methanolic solution [45]. In this compound the neutral units $Cu(thiochrome)Cl_2$ are linked together through N(1) and N(3) atoms of two thiochrome molecules forming an infinite chain. In 1991 Kitagawa and co-workers synthesized a polymeric structure with formula $Cu_2(thiochrome)_2(ClO_4)_2$, using $Cu(ClO_4)_2{\cdot}6(H_2O)$ and thiochrome in methanolic solution [46]. This compound contains Cu ion in the +1 oxidation state and has a three-coordinate T-shaped geometry with two nitrogen donors N(2) and N(3) of two different thiochrome ligands and one oxygen donor O(1) forming a dimeric copper units $[Cu_2(thiochrome)_2]^{2+}$ which are linked by the hydroxyl group of the coordinated thiochrome forming infinite polycation chains with anion columns of ClO_4 along these chains.

Here we wish to report the synthesis and structural characterisation of two copper (I) compounds of thiochrome and to explore their behaviour in the colorectal cancer cell line Caco-2. To the best of our knowledge, no cytotoxicity studies have been performed with copper (I) thiochrome compounds.



Scheme 2 Different coordination modes of thiochrome (M=metal)

2. Experimental section

2.1 Materials and measurements

Copper(II) acetate (Riedel-de Haën), thiamine hydrochloride (Riedel-de Haën) were all of analytical grade and used as received.. Elemental analysis was performed on a Truspec 630-200-200 analyzer. FT-IR spectra were recorded on a Mattson 7000 spectrophotometer with KBr pellets in the range of 4000-400 cm^{-1}. The diffuse reflectance of solid complexes (UV-vis) was recorded at room temperature on a Jasco V560. The absorption UV-vis spectra in DMSO solution was measured at room temperature on a Cintra 303 GBC ^{1}H-NMR spectra were recorded with Bruker DRX 300 spectrometer (300 MHz for ^{1}H), in DMSO-d_6 as solvent.

Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz; internal standard was residual peak of the solvent.

2.2 Synthesis

Synthesis of compound **1** $\{Cu_4[(thiochrome)_2Cl_4]\cdot 2(H_2O)\}_n$ and compound **2** $Cu_2[(thiochrome)_2Cl_2]$*:* A mixture of copper(II) acetate (1.871 mmol, 0.452 g), and thiamine hydrochloride (1.868 mmol, 0.230 g) in 25 ml of water was stirred for 2 h at room temperature. After that the biker with this solution was transfer for other big biker with acetone, which was covered with parafilm paper containing smalls walls allowing the slow evaporation. After 2-3 days a yellow rod crystals **1** and yellow small needles **2**, suitable for single X-ray analysis were obtained, with a ratio of 30 to 70% respectively. The two compounds crystalize from the same solution, showing different crystal size consequently it was easier separate them by filtration and washing with distilled water. The bigger crystals (compound 1) stay on the top of the filter and the second phase (compound 2) pass through the filter. In a second step the second phase was filtered using a very small pore size filter to retain on the top the compound 2. After that both compounds were dry at room temperature. Elemental analysis (%) for compound **1**, $C_{24}H_{32}Cl_4Cu_4N_8O_4S_2$: Calc. C, 30.11; H, 3.35; N, 11.71; Found: C, 31.38; H, 3.22; N, 12.04.

Elemental analysis (%) for compound **2** $C_{24}H_{28}Cl_2Cu_2N_8O_2S_2$: Calc. C, 39.85; H, 3.87; N, 15.50; Found: C, 40.81; H, 3.97; N, 14.63.

The IR spectrum of compound **1** show the ν(C=C) and ν(C=N) vibrations of the heterocyclic ring as bands between 1606 and 1333 cm^{-1}. The band at 3302 cm^{-1} is attributed to OH vibrations from thiochrome hydroxyl group and from the water molecules. The weak band at 469 cm^{-1} can be attributed to Cu-N vibrations (see figure S1).

The IR spectrum of compound **2** show the ν(C=C) and ν(C=N) vibrations of the heterocyclic ring as a bands between 1587 and 1332 cm^{-1}. The band at 3265 cm^{-1} is attributed to OH vibrations from thiochrome hydroxyl group. The bands between 492 and 442 cm^{-1} can be attributed to Cu-N vibrations (see figure S2).

2.3 Crystal structure determination

The X-ray single crystal data of all complexes were collected with monochromated Mo-Kα radiation (λ = 0.71073 Å) on a Bruker SMART Apex II diffractometer equipped with a CCD area detector at 150(2) K for compounds **1** and **2**. The crystals were positioned at 40 mm from the CCD and the spots were measured using 60 s counting time. Data reduction was carried out using the SAINT-Plus software package [47]. Multi-scan absorption correction was applied to all intensity data using the SADABS program [48]. The structure was refined *via* full matrix least squares on F^2 using the SHELX-2013 suite [49].

All non-hydrogen atoms were refined with anisotropic thermal displacements. The C-H hydrogen atoms were included at calculated positions and refined with isotropic parameters equivalent to 1.2 times those of the atom to which they are attached. The hydrogen atoms bonded to coordination waters were obtained from

the last final difference Fourier maps and refined with O…H distance restraints consistent with a tetrahedral geometry. Molecular diagrams were drawn with Olex2 software [50].

The crystallographic data for both copper compounds are listed in Table S1. CCDC (compound 1- 1825833 and compound 2- 1825832) contains the supplementary crystallographic data for this paper.

2.4 In vitro cytotoxicity studies

2.4.1. Caco-2 cell culture

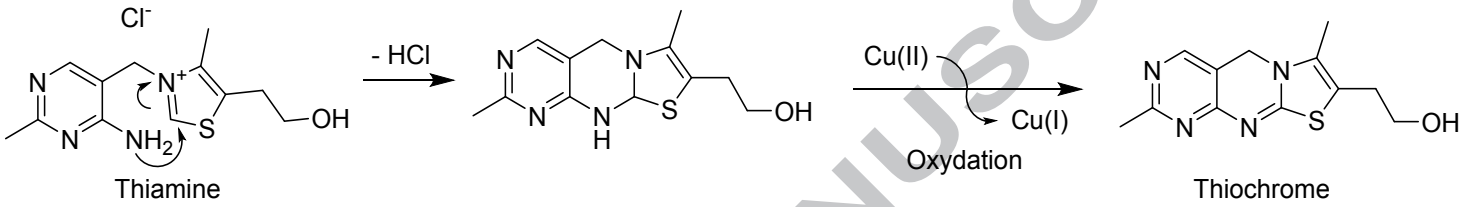
The Caco-2 colorectal adenocarcinoma cell line was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and was used between passages numbers 43–47. The cells were maintained in a humidified atmosphere of 5% CO_2/95% air and were cultured in Minimum Essential Medium containing 5.55 mM glucose, 15% fetal calf serum, 25 mM HEPES, 100 units/mL penicillin, 100 mg/mL streptomycin and 0.25 mg/mL amphotericin B (all from Sigma). The culture medium was changed every 2–3 days and the culture was split every 7 days. For subculturing, the cells were removed enzymatically (0.25% trypsin-EDTA, 5 min, 37 °C), split 1:3, and subcultured in plastic culture dishes (21 cm^2; Corning Costar, Corning, NY, USA). For the experiments, cells were seeded on 24-well plastic cell culture clusters (1.9 cm^2; TPP®, Trasadingen, Switzerland), and the experiments were performed 7–9 days after the initial seeding.

2.4.2 MTT assay

The capacity to interfere with the growth of Caco-2 cells was determined by the MTT (3,4,5-dimethylthiazolyl-2-2,5-diphenyl-tetrazolium bromide; Sigma) method [51]. Briefly, after a 24h-exposure to compounds to be tested (compound **1** and **2**, thiamine, thiochrome and N-acetylcysteine), 50 µL of MTT solution (5 mg/mL) was added to each well and the cells were further incubated for 3h at 37°C. Afterwards, the MTT solution was removed and the cells were lysed by the addition of 200 µL of dimethylsulfoxide followed by plate shaking for 10 min at room temperature. Optical density for the solution in each well was determined at both 540 and 660 nm. OD at 660 nm corresponds to non-specific light absorption and was subtracted from the OD at 540 nm to give the OD value specific to formazan crystals derived from MTT cleavage, which is proportional to the number of viable cells with active mitochondria. The IC_{50} values were calculated by nonlinear regression analysis using the Graphpad Prism software, with sigmoidal dose-response curve fitting [52].

3. Results and discussion

Both copper compounds **1** and **2** contain thiochrome produced by oxidation of thiamine by copper(II) at room temperature. Thiochrome can indeed be conveniently synthesized by cyclization and oxidation of thiamine (vitamin B_1). This transformation has been described in the presence of a base (potassium carbonate) and an oxidant (iodine) [53]. We observed a similar transformation during the synthesis of compounds **1** and **2**, with the acetate playing the role of the base and the copper (II) of the oxidant (scheme 3).



Scheme 3 Proposed mechanism for the formation of thiochrome and copper (I) in situ.

The X-ray crystal structures of both compounds were well resolved for data collected at 150 K. Selected bond distances and angles around the copper centres are gathered in table 1 and the hydrogens bonds are illustrated in table 3. The purity of each compound can be checked by comparison of the experimental X–ray powder diffraction pattern with the powder pattern calculated from the structure solved from single-crystal X-ray diffraction data (figures S3 and S4)

Table 1

Bond distances (Å) and angles (º) for compounds **1** $\{Cu_4[(\mu_3\text{-thiochrome})_2Cl_4]\cdot 2(H_2O)\}_n$ and **2** $Cu_2[(\mu_2\text{-thiochrome})_2Cl_2]$

Bond lenghts		**Bond angles**	
Compound 1			
Cu(1)-N(1)	2.0053(14)	N(1)-Cu(1)-Cl(1)	136.06(4)
Cu(1)-Cl(1)	2.2211(5)	N(1)-Cu(1)-Cl(2)	107.27(4)
Cu(1)-Cl(2)	2.3184(5)	Cl(1)-Cu(1)-Cl(2)	116.43(2)
Cu(2)-N(2i)	1.9497(14)	N(2i)-Cu(2)-N(3)	156.69(6)
Cu(2)-N(3)	1.9409(14)	N(2i)-Cu(2)-Cl(1ii)	103.71(4)
Cu(2)-Cl(1ii)	2.4733(5)	N(3)-Cu(2)-Cl(1ii)	99.29(5)
Cu(2)-Cu(2i)	2.5383(4)		
Compound 2			
Cu-N(2)	1.967(4)	N(2)-Cu-N(3iv)	154.94(17)
Cu-N(3iv)	1.962(2)	N(2)-Cu-Cl	102.66(12)
Cu-Cl	2.3910(14)	N(3iv)-Cu-Cl	101.25(13)
Cu-Cu(iv)	2.5984(12)		

i=1-x,2-y,1-z; ii=1+x,y,z; *iv=1-x, 1-y,1-z;*

3.1 Crystal structure of $\{Cu_4[(thiochrome)_2Cl_4]\cdot 2(H_2O)\}_n$ 1

The reaction between copper acetate, thiamine hydrochloride in water at room temperature produced, after 3 days of slow evaporation, a yellow rod crystals (compound **1**) and a yellow powder (small needles-compound **2**) suitable for single crystal X-ray diffraction.

Single crystal X-ray diffraction reveals that compound **1** formulated as$\{Cu_4[(thiocrome)_2Cl_4]\cdot 2(H_2O)\}_n$ exhibits a 1D polymeric structure along *a* direction (figure 1a). The asymmetric unit contains two crystallographically independent copper(I) centres, one thiochrome ligand and one water solvent molecule. Both Cu(I) centres adopt a distorted trigonal pyramidal geometry (figure 1b). The Cu(1) ion is coordinated by nitrogen N(1) atom from thiochrome (Cu(1)-N(1)=2.0053(14) Å) and two chloride atoms (Cu-Cl(1)=2.2211(5) Å; Cu-Cl(2)=2.3184(5) Å (see table 1). On the other hand, Cu(2) coordinates thiochrome *via* N(2i) (Cu2-N2i=1.9497(14) Å) and N(3) (Cu(2)-N(3)=1.9409(14) Å) nitrogen atoms from two thiochrome molecules forming a dimer with Cu(2)…Cu(2i) distance of 2.5383(4) Å, and one Cl atom (Cu(2)-Cl(1)=2.4733(5) Å). Interestingly Cu(1) atoms are linked with Cu(2) centres through Cl(1) by vertex sharing (figure 1b) exhibiting metal chain of type Cl(2)-Cu(1)-Cl(1)-Cu(2)-Cu(2)-Cl(1)-Cu(1) with Cu(1)…Cu(2) distances of 3.081 (4) Å and also linked by a thiochrome ligand forming an infinite chain along *a* direction. The Cu-N (1.9409(14)-2.0053(14) Å) distances compare well with those observed for the two compounds with thiochrome described in literature [45, 46], in which the Cu centers are assembled by thiochrome bridging ligand. Compound **1** show a similar dimeric copper structure to the one found for compound $\{Cu_2(thiochrome)_2(ClO_4)_2\}_n$ with metal distance of 2.5383(4) Å and 2.476(3) Å, respectively, these distances are imposed by the μ-N(2)–N(3) coordination mode of the thiochrome bridge ligand. The Cu-Cl (2.2211(5)-2.4733(5) Å) bond distances are a bit longer than those found for compound $\{Cu(thiochrome)Cl_2\}_n$ (Cu-Cl=2.216 and 2.267 Å), however there is one copper(I) complex of thiamine $Cu(thiamine)Cl_2$ prepared by Cramer *et al.* [25], in which Cu-Cl=2.337 Å.

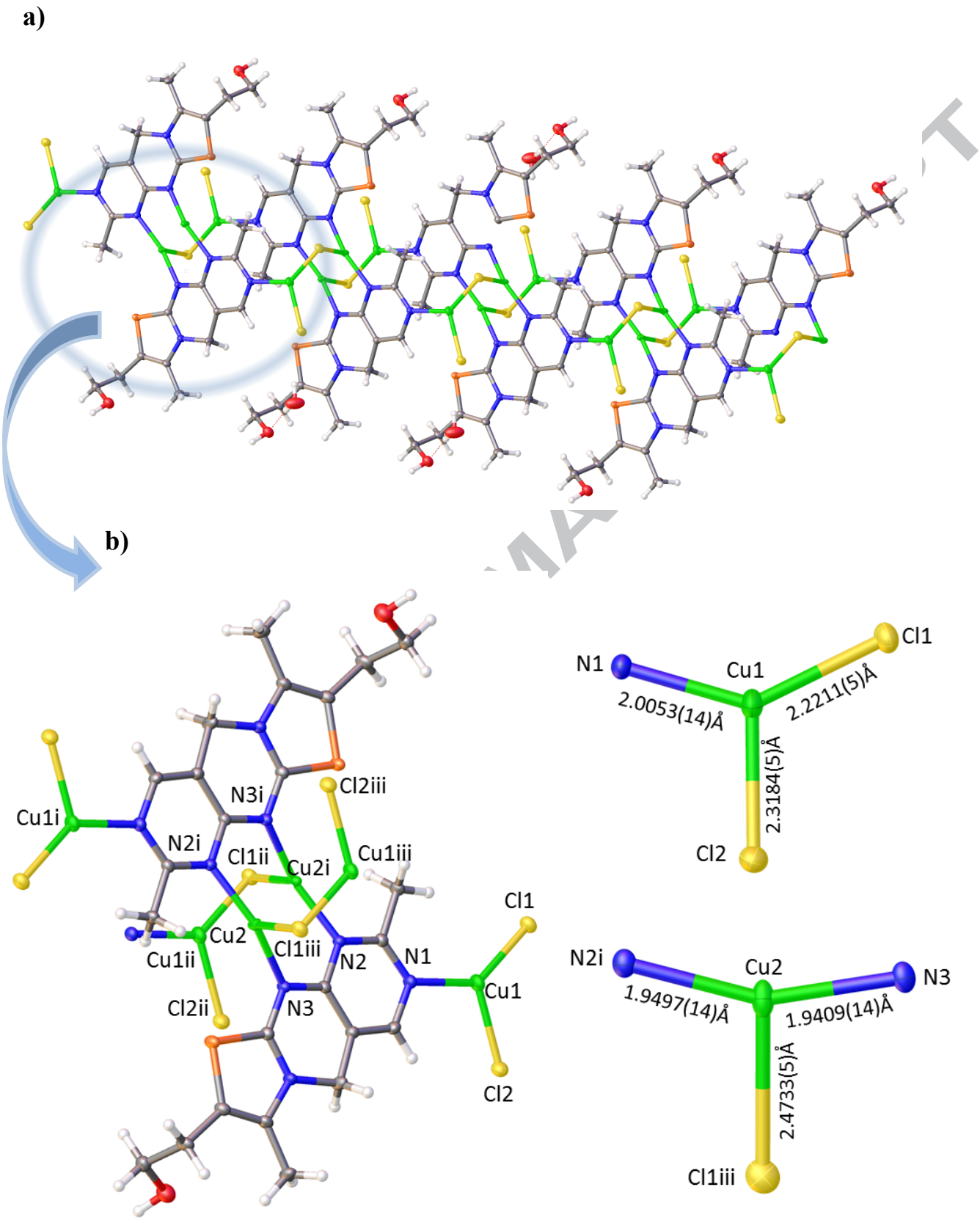


Fig. 1 **a)** 1D layer of $\{Cu_4[(\mu_3\text{-thiochrome})_2Cl_4]\cdot 2(H_2O)\}_n$ **1** viewed along [001] direction **b)** left: Detailed of 1D layer with relevant atomic notation scheme; right: copper centres environment. Colour scheme: Cu-green; Cl-yellow; S-orange; O-red; N-blue; C-gray; H- white. *i=1-x,2-y,1-z; ii=1+x,y,z; iii=-x, 2-y, 1-z*

The O(100)···O(1) and O(100)···Cl(2) intermolecular distances found in the crystal lattice between the oxygen atom from the coordination water molecule and the oxygen from the hydroxyl group of thiochrome as well with the chloride atom (table 2) are consistent with the existence of a 2-D dimensional network of O-H···O and O-H···Cl hydrogen bonding (see figure 2).

Table 2

Hydrogen bond dimensions for compounds **1** $\{Cu_4[(\mu_3\text{-thiochrome})_2Cl_4]\cdot 2(H_2O)\}_n$ and **2** $Cu_2[(\mu_2\text{-thiochrome})_2Cl_2]$

D-H···A	H···A/ Å	D···A/ Å	D-H···A/º
Compound 1			
O(1)-H(1)…Cl(2) [*1-x*,1-*y*,2-z]	2.18(4)	3.1268(16)	167(4)
O(100)-H(10D)…Cl(2) [*1+x*,*y*,z]	2.512(17)	3.2802(19)	150.9(18)
O(100)-H(10E)…O(1)	2.00(4)	2.836(3)	170(4)
Compound 2			
O(1)-H(1)…N(1) [3/2+*x*,1/2-*y*,-1/2+z]	2.05(7)	2.843(5)	158(7)

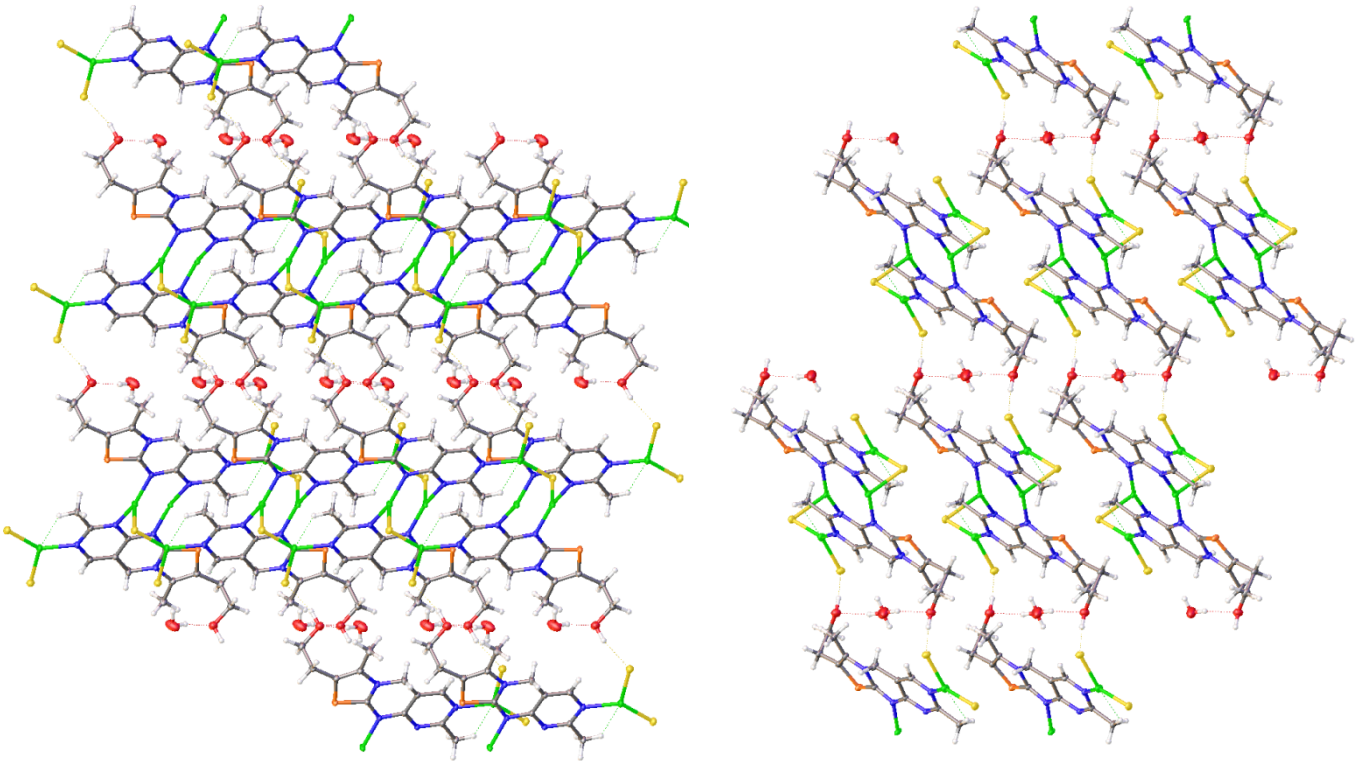
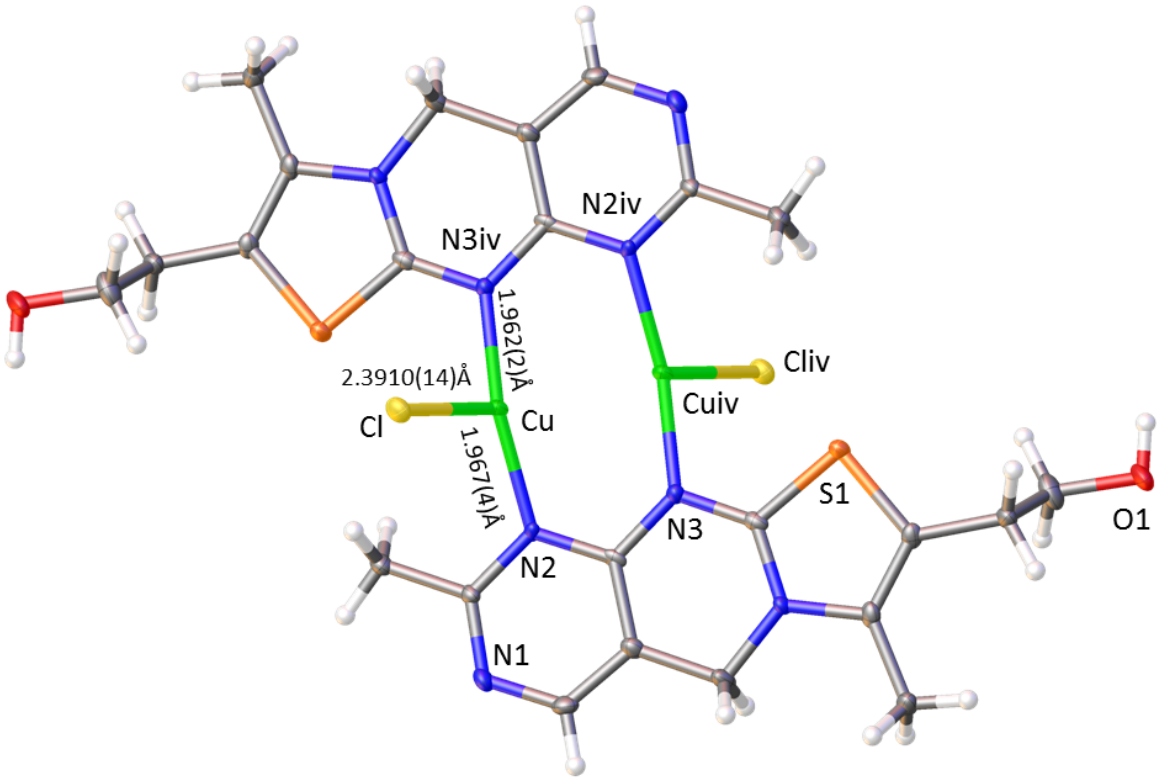


Fig. 2 Packing diagrams of compound **1**; left) viewed along [001] direction; right) viewed along [100] direction; O-H···O bonding interactions are drawn as red dashed lines.

3.2 Crystal structure of $Cu_2[(\mu_2\text{-thiocrome})_2Cl_2]$ **2**

Small yellow needles of copper thiochrome compound were obtained and its structure determined by single crystal X-ray diffraction. A molecular diagram of dinuclear compound **2**, $Cu_2[(\mu_2\text{-thiocrome})_2Cl_2]$ with thermal ellipsoids drawn at 50% level and relevant atomic notation scheme is presented in Fig. 3. Selected bond distances and angles around the copper(I) centers are gathered in Table 1. Compound **2** comprises a dimeric copper structure linked through N(2) and N(3) from thiochrome ligand exactly as found in compound **1** described previously. This compound crystallizes in the space group $P2_1/n$ with half a molecule in the asymmetric unit. The two halves of the molecule are related by an inversion centre between the copper ions at the centre of mass of the complex. The metal distance Cu···Cu of 2.5984(12) Å is comparable to that observed in compound **1** (2.5383(4) Å) as well as the bond distances Cu-N and Cu-Cl (table 2).



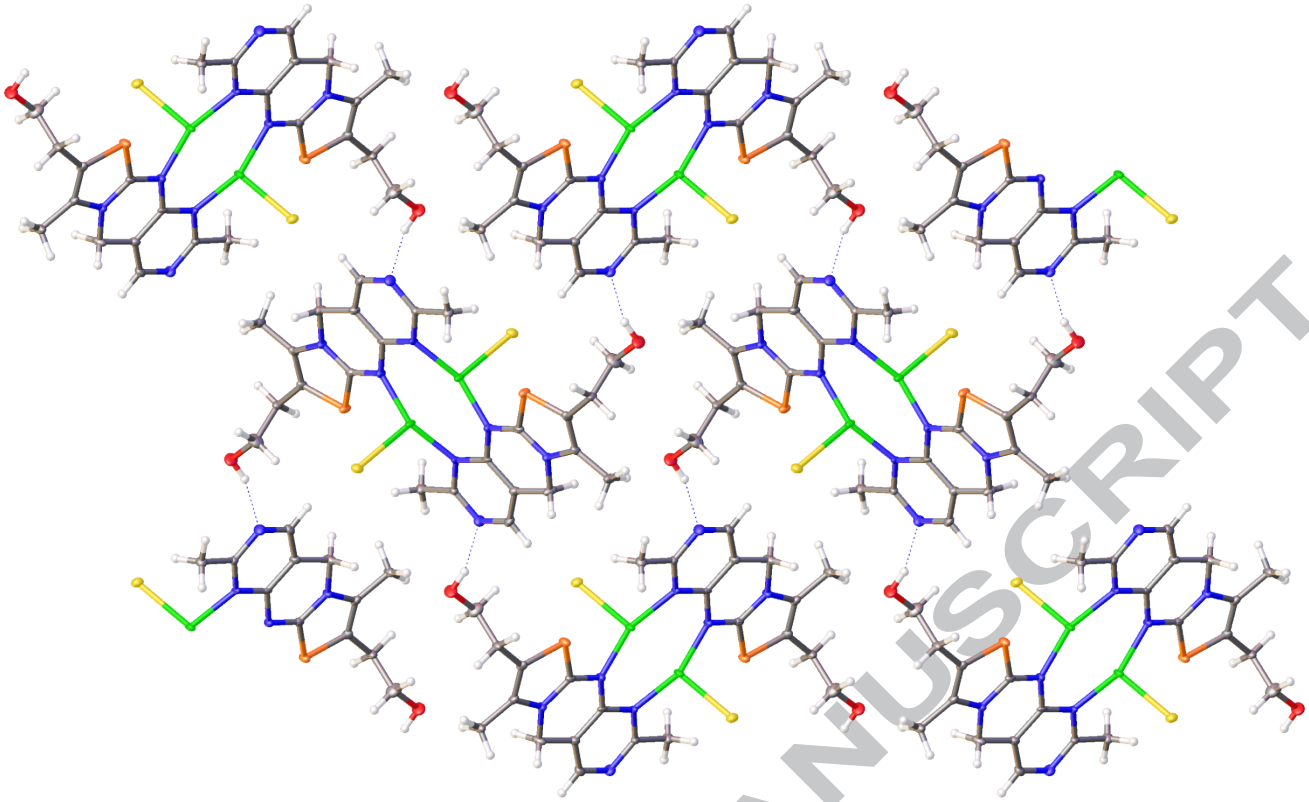


Fig. 3 top) Molecular diagram of $Cu_2[(\mu_2\text{-thiocrome})_2Cl_2]$ **2**; **down)** Crystal packing viewed along [100] direction.O-H···N hydrogen bonds drawn as blue dashed lines. Colour scheme: Cu-green; Cl-yellow; S-orange; O-red; N-blue; C-gray; H- white. *iv=1-x, 1-y,1-z;*

The crystal structure of compound **2** is stabilized by a 2-D network of O-H···N hydrogen bonds in which $Cu_2[(thiocrome)_2Cl_2]$ units are assembled through hydroxyl group from one thiochrome ligand as donor and the adjacent N(1) atom as acceptor with O···N distance of 2.843(5) Å, (Figure 2b). The geometric parameters of the hydrogen bonds are listed in table 3.

3.3 Physical characterization of the compounds 1 and 2

3.3.1 Ultraviolet-Visible (UV_vis) Spectroscopy

Compounds **1** and **2**, as described in section 3.1 and 3.2, contains Cu(I) ions showing yellow color. The UV_vis spectra in solid phase of the two compounds and thiochrome could be observed in figure 4, The three compounds show similar behavior a broad and intense band centered at ca. 410 nm, which may be attributed to ligand-to-ligand charge-transfer (LLCT) transitions. No bands due to d-d transitions are observed which is consistent with the presence of Cu(I) ions.

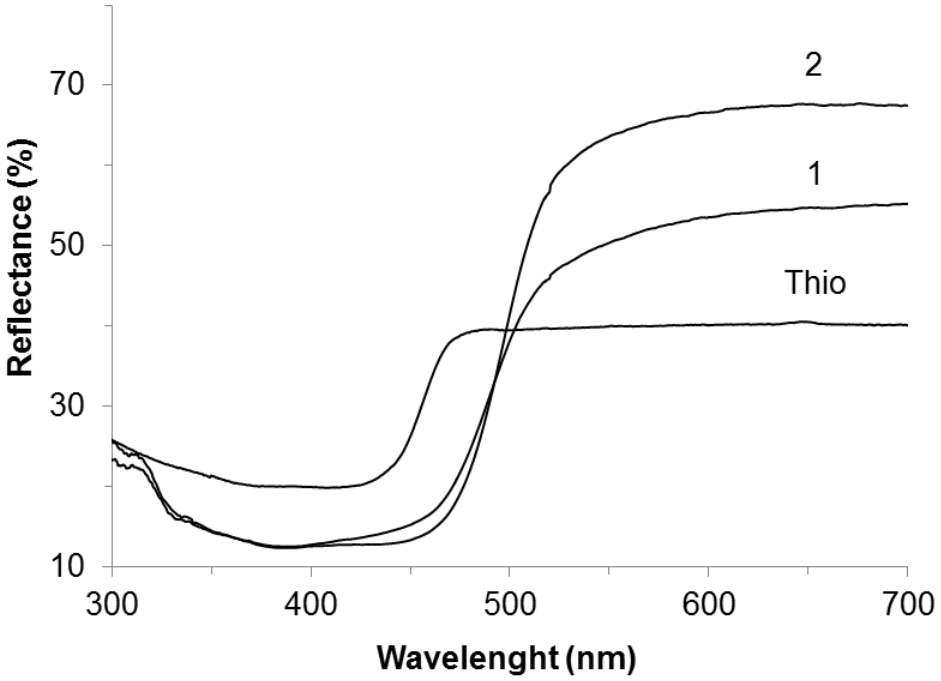


Fig. 4 UV-vis spectra in solid phase of copper compounds 1, 2 and the thiochrome ligand

In figure 5 we present the comparison of UV-vis spectra of compounds 1, 2 and the thiochrome ligand in DMSO solution. All the three samples exhibit an absorption band centred at 412 nm for thiochome, 405 nm for complex 2 and at 396 nm for compound 2, which could be assigned to ligand-to-ligand charge-transfer (LLCT) transitions. The absence of the absorption bands in the visible region indicate that in DMSO solution Cu(I) is not oxidized to Cu(II)

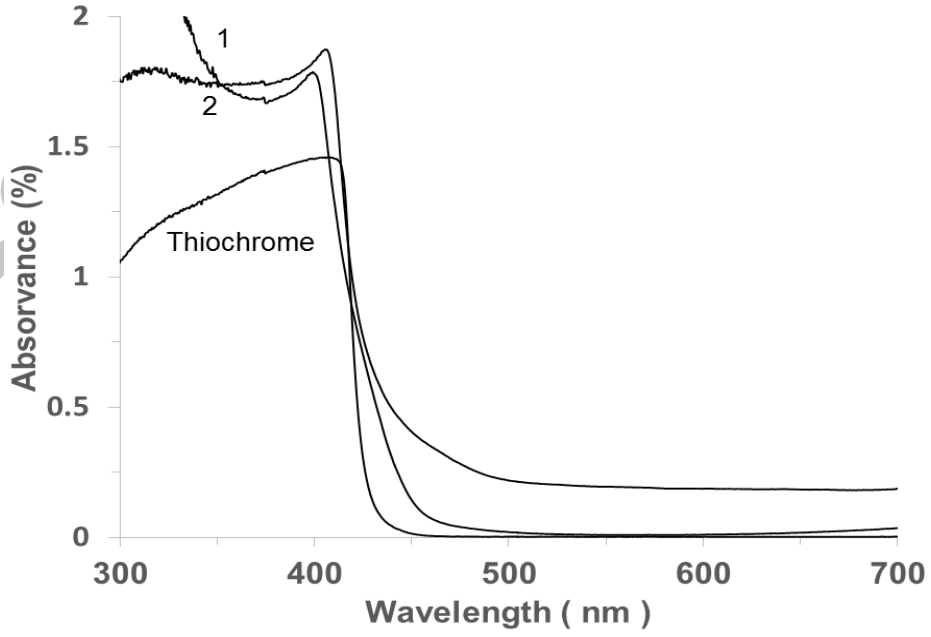


Fig. 5 UV-vis spectra in liquid phase of copper compounds 1, 2 and the thiochrome ligand

3.3.2 *NMR Spectroscopy*

The ^{1}H NMR spectra in DMSO-d_6 of thiochrome and compounds **1** and **2**, are shown in figure 6. For thiochrome, the signals of the aromatic proton, the methylene bridge, and the propyl chain appear around 8.0 ppm, 5.5 ppm and 5.0, 3.5 and 2.7 ppm, respectively. The obtention of NMR spectra for compounds **1** and **2** rules out the presence of Cu (II). All peaks corresponding to thiochrome are present in the spectra of the complexes, but they are shifted when compared to pure thiochrome, illustrating the presence of a complex, most probably of Cu(I). The peaks are well defined, which also rules out the presence of polymeric material. These NMR spectra support the presence of complexes of thiochrome with Cu(I) in solution, but do not allow determination of their exact stoichiometry nor of their exact structure.

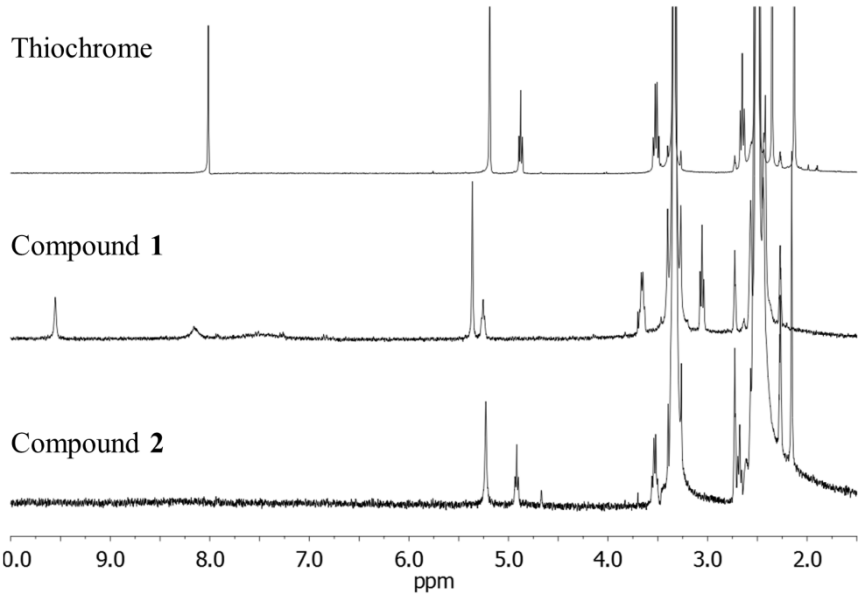


Fig. 6 ^{1}H NMR of compounds **1**, **2** and thiochrome in DMSO-d_6

3.4 In vitro cytotoxicity assays

As shown in Fig. 7, both compounds showed a concentration-dependent cytotoxic effect against Caco-2 cells, with a similar potency (IC_{50} values of 146 and 191 μM, respectively). Interestingly, both compounds were much more potent than thiamine and thiochrome (Fig. 8). In order to investigate the mechanism responsible for the cytotoxic effect of compound **1** and **2**, we tested the putative involvement of changes in oxidative stress levels. As shown in Fig. 9, ROS scavenging did not change the effect of compound **1**, and it only slightly inhibited the effect of compound **2**. So, oxidative stress do not appear to play an important role in the cytotoxic effect of compound **1** and **2**.

Comparison between the cytotoxic activity of our compounds and other anticancer drugs shows that compound **1** and **2** are slightly more potent than cisplatin in this cell line (IC_{50} for cisplatin = 274±90 μM)

[19, 20]. Recently, we reported a copper(I) polymeric structure compound with some similarities to compound **1** (although the organic ligand is vitamin B_3 $[Cu(\text{vitamin } B_3)Cl]_n$ instead of vitamin B_1) and this compound presented an IC_{50} of 206 (163-267) μM in relation to Caco-2 cells viability [20]. These results suggest that the cytotoxicity of these compounds may be attributed to the electronic properties of Copper(I) centers and not so much to the organic linker. Based on this assumption, investigation on binding ability of the copper(I) compounds to DNA is in progress. The reported cu(I) complexes [15-18], show significant improved anticancer activity for several cell lines compared to cisplatin, on basis of these results the Cu(I) thiochome compounds described in this paper showed mild antiproliferative effects against Caco-2 cells.

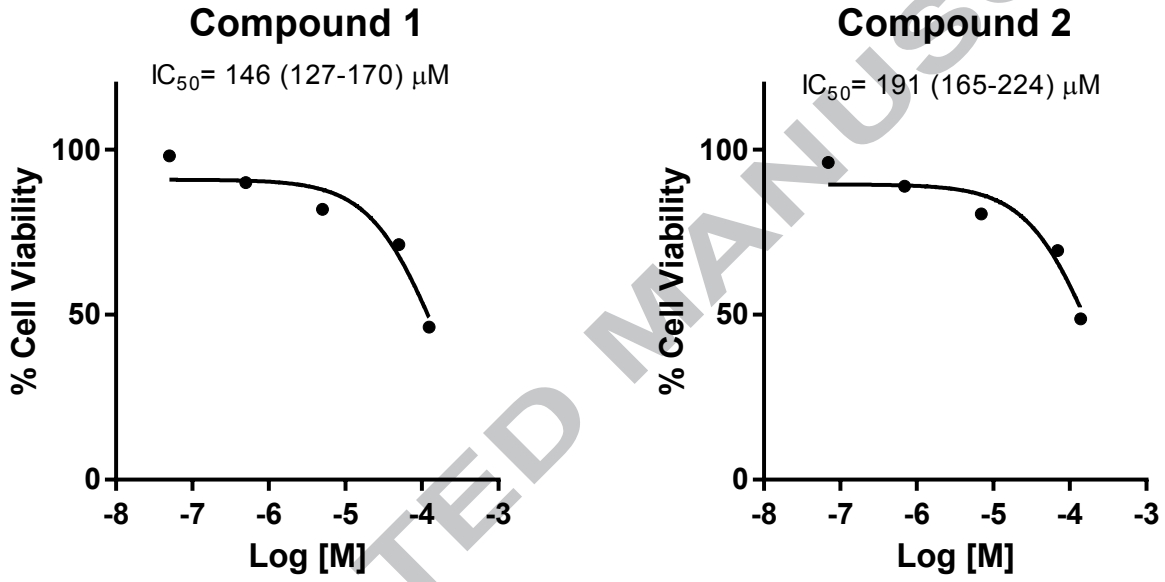


Fig. 7 IC_{50} values for compounds **1** and **2** cytotoxicity as a function of their concentration, against Caco-2 cells. Shown are arithmetic means ± SEM. IC_{50} values are presented as geometric means (with 95% confidence intervals) (n = 6–9).

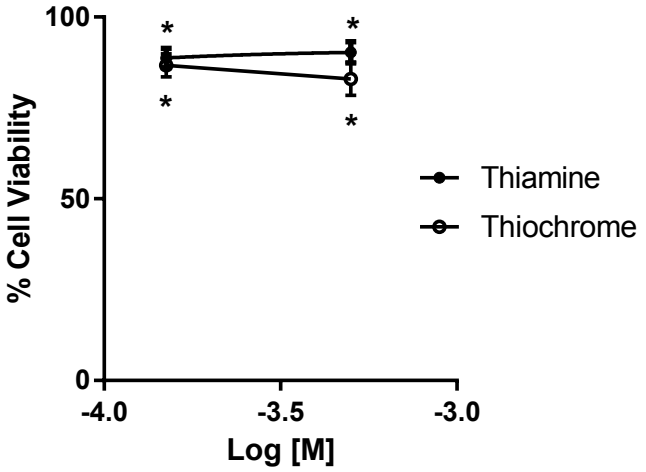


Fig. 8 Cytotoxicity of thiamine and thiochrome, against Caco-2 cells. Shown are arithmetic means ± SEM. * significantly different from control (Student't t test) (n = 8).

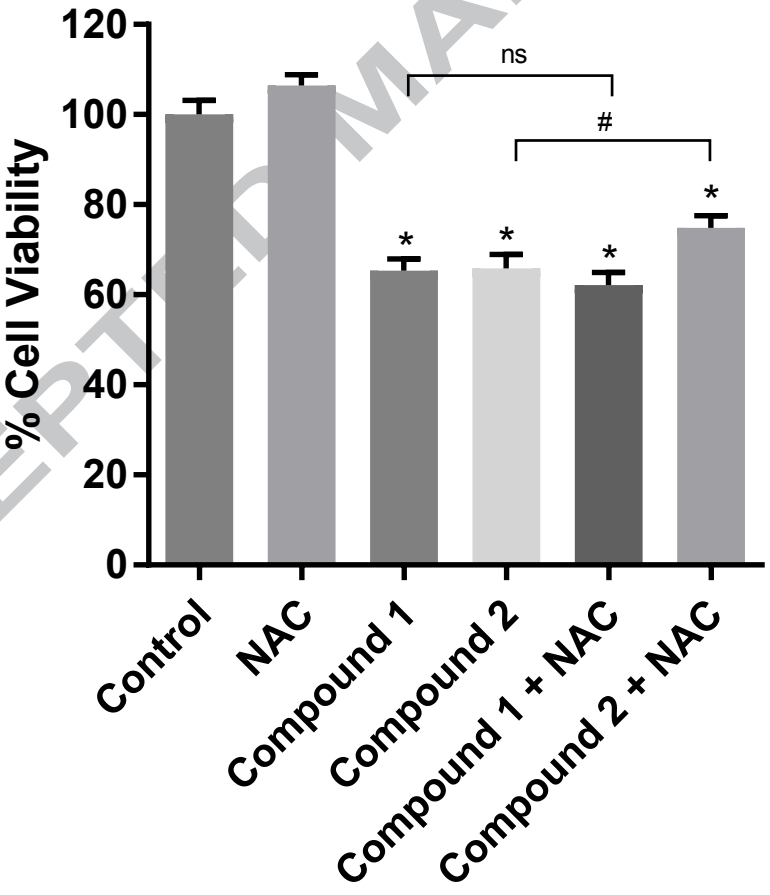


Fig. 9 Interference of the ROS scavenger N-acetylcysteine (NAC) with the cytotoxic effect of compound **1** and **2**, against Caco-2 cells. Shown are arithmetic means ± SEM. * significantly different from control (Student't t test), # significantly different from each other, ns no significantly different from each other (ANOVA followed by Student-Newman-Keuls test) (n = 8).

4. Conclusions

In conclusion, our results indicate that two new Cu(I) compounds with thiochrome were synthetized at room temperature by the reaction of copper acetate and thiamine hydrochloride in water, making them low cost and environmentally friendly compounds. For both compounds, thiochrome binds the copper centers in a bridged mode through the N(2) and N(3) atoms and also through N(1) for compound **1**, generating a polymeric and dimeric crystal structures, respectively, with short Cu···Cu distances (2.476(3), 2.5383(4) and 2.5984(12) Å). The biological studies carried out with human colon adenocarcinoma Caco-2 cells showed that both compounds decrease the viability of these cells, much more potently than thiamine or thiochrome, and that changes in oxidative stress levels do not appear to mediate the cytotoxic effect of the compounds. It would be important to further investigate the mechanism of action involved in the cytotoxic effect of these compounds, as well as their effect in other cellular characteristics such as apoptosis, cell proliferation and migration, their effect in other colon and also in non-colon cancer cell lines and their effect in non-cancer cell lines. Further investigation concerning the synthesis of other mononuclear and multinuclear Cu(I)/Cu(II) thiochrome compounds as well as Cu(I)/Cu(II) thiamine compounds for comparison and to make a relationship structure/cytotoxicity, are currently underway. In this sense, this type of cheap, biocompatible and environment friendly compounds may be a starting point to develop potentially interesting anticancer drugs.

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Supplementary material

†Electronic Supplementary Information (ESI) available: CCDC 1825833 and 1825832 for compounds 1 and 2, contains the supplementary crystallographic data for this paper. Copy of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK fax (+44)1223 336033, e-mail: deposit@ccdc.cam.ac.uk

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List of figures and tables

Table 1 Bond distances (Å) and angles (º) for compounds **1** $\{Cu_4[(\mu_3\text{-thiochrome})_2Cl_4]\cdot 2(H_2O)\}_n$ and **2** $Cu_2[(\mu_2\text{-thiocrome})_2Cl_2]$

Table 2 Hydrogen bond dimensions for compounds **1** $\{Cu_4[(\mu_3\text{-thiochrome})_2Cl_4]\cdot 2(H_2O)\}_n$ and **2** $Cu_2[(\mu_2\text{-thiocrome})_2Cl_2]$

Fig. 1 a) 1D layer of $\{Cu_4[(\mu_3\text{-thiochrome})_2Cl_4]\cdot 2(H_2O)\}_n$ **1** viewed along [001] direction **b)** left: Detailed of 1D layer with relevant atomic notation scheme; right: copper centres environment. Colour scheme: Cu-green; Cl-yellow; S-orange; O-red; N-blue; C-gray; H- white. *i=1-x,2-y,1-z; ii=1+x,y,z; iii=-x, 2-y, 1-z*

Fig. 2 Packing diagrams of compound **1**; left) viewed along [001] direction; right) viewed along [100] direction; O-H···O bonding interactions are drawn as red dashed lines.

Fig. 3 top) Molecular diagram of $Cu_2[(\mu_2\text{-thiocrome})_2Cl_2]$ **2**; **down)** Crystal packing viewed along [100] direction.O-H···N hydrogen bonds drawn as blue dashed lines. Colour scheme: Cu-green; Cl-yellow; S-orange; O-red; N-blue; C-gray; H- white. *iv=1-x, 1-y,1-z;*

Fig. 4 UV-vis spectra in solid phase of copper compounds 1, 2 and the thiochrome ligand

Fig. 5 UV-vis spectra in liquid phase of copper compounds 1, 2 and the thiochrome ligand

Fig. 6 ^{1}H NMR of compounds **1**, **2** and thiochrome in DMSO-d_6

Fig. 7 IC_{50} values for compounds **1** and **2** cytotoxicity as a function of their concentration, against Caco-2 cells. Shown are arithmetic means ± SEM. IC_{50} values are presented as geometric means (with 95% confidence intervals) (n = 6–9).

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Highlights

Development of novel Cu(I) compounds with vitamin B_1 derivative and their potential application as anticancer drugs

Paula Brandão[a]*, Samuel Guieu[a,b], Ana Correia-Branco[c], Cláudia Silva[c], Fátima Martel[c]*

[a]Departamento de Química, CICECO, Universidade de Aveiro, 3810-193 Aveiro, Portugal.

[b] Departamento de Química,QOPNA, Universidade de Aveiro, 3810-193 Aveiro, Portugal.

[c]Departamento de Biomedicina, Unidade de Bioquímica, Faculdade de Medicina do Porto e I3S, Universidade do Porto, 4200-319, Porto, Portugal

* Corresponding authors e-mail address: pbrandao@ua.pt; fmartel@med.up.pt

1 – Two copper (I) thiochrome (vitamin B_1 derivative) compounds were synthesized at room temperature.

2 - The structures were determined by single crystal X-ray diffraction.

3 – The biological studies carried out with human colon adenocarcinoma Caco-2 cells showed that both compounds decrease the viability of these cells.