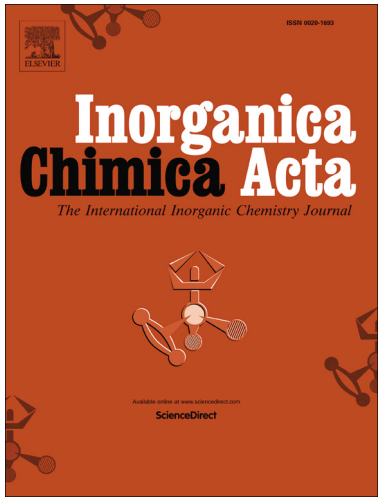


Research paper

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# New copper(II) niflumate complexes with N-donor ligands: synthesis, characterization and evaluation of anticancer potential against human cell lines

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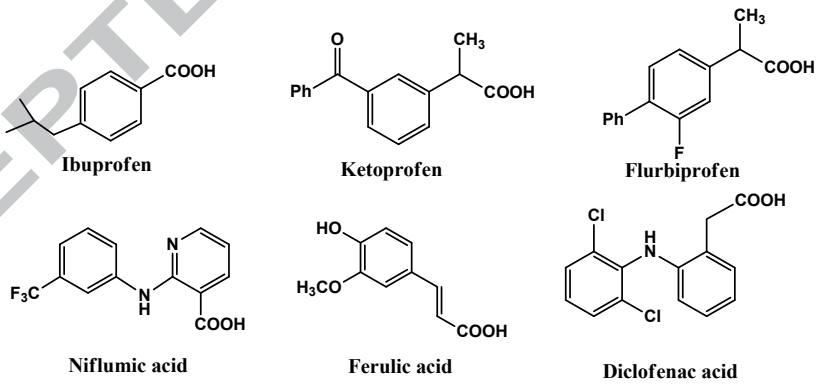
**Abstract**

Five new copper(II) niflumate complexes, [Cu(*en*)$_2$(*nif*)$_2$], **1**, [Cu(unsym-*dmen*)(*nif*)$_2$], **2**, [Cu(p*n*)$_2$(*nif*)$_2$][Cu(*pn*)$_2$(H$_2$O)$_2$](*nif*)$_2$ **3**, [Cu(β-pic)$_2$(*nif*)$_2$], **4**, [Cu(γ-pic)$_2$(*nif*)$_2$] **5** (where *en* =ethylenediamine, *pn* = propan-1,3-diamine, *unsym-dmen* = unsymmetrical 1,1-dimethyl-ethylenediamine, *β-pic* = 3-methylpyridine, *γ-pic* = 4-methylpyridine, nif = niflumate) have been synthesized by using appropriate starting materials and methanol-water (4:1 v/v) as solvent. All the above synthesized complexes **1-5** have been characterized by spectroscopic methods (UV-Vis, FT-IR, and EPR). The structures of complexes **1, 3** and **5** have been unambiguously determined by single crystal X-ray structure determination which clearly revealed that these complexes are mononuclear complexes. The structure of complex **4** has been optimized by DFT calculations. In all complexes **1-5**, niflumate shows direct coordination to central copper(II) ion leading to covalent character of all complexes, although the complex **3** also contains cationic moiety [Cu(p*n*)$_2$(*nif*)$_2$]$^{2+}$ and two niflumate ions. The ability of all the complexes to show cytotoxicity against human cancer cell lines has also been evaluated.

**Keywords**: copper(II) complexes; niflumic acid; NSAIDs; cytotoxicity; cancer cell lines

## 1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are such an important class of drugs, capable of alleviating pain, inflammation and fever in both humans and animals (with tolerable side effects), that they are available from a chemist shop without a medical prescription. What is more remarkable about them is that they can also show significant antitumor activities either by themselves (cancer cell death by apoptosis) or through a mechanism involving free radicals or in synergistic combination with other antitumor agents [1-4]. Even though much diversification exists within NSAIDs and sub-classifications are possible (*e.g.*, salicylates, oxicams, furanones, fenamates *etc.*), a commonality that can be traced among most of them is the presence of an arylcarboxylate group [4-6]. A typical and important example is niflumic acid, (2-{[3-(trifluoromethyl)phenyl]amino}-3-pyridine-carboxylic acid, H*nif*), which acts like an enzyme inhibitor against the prostaglandin-producing cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes [6-9]. Other very commonly used NSAIDs against COX-1 and COX-2 which possess an arylcarboxylate group are shown in Scheme 1.



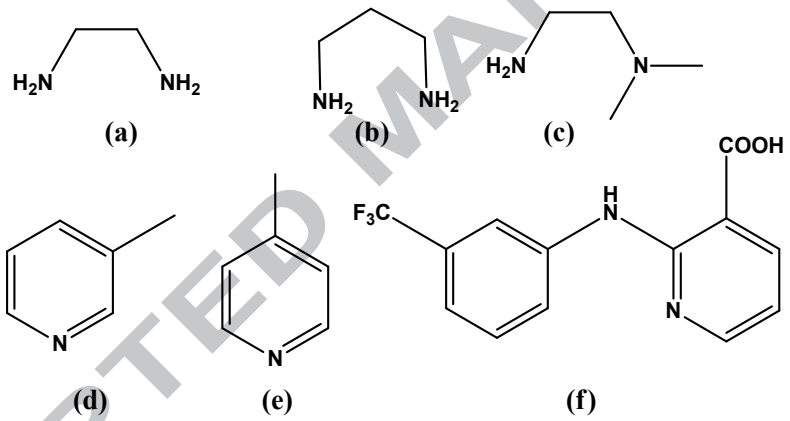

**Scheme 1**. Structural formulae of some commonly used NSAIDs [4, 6]

Besides being an important class of NSAIDs, arylcarboxylates are also equally important class of ligands in bioinorganic chemistry that exhibit a wide range of coordination modes and associated geometries. Once deprotonated, the arylcarboxylate anion such as niflumate (*nif*) can

have four lone pairs of electrons available for metal binding. Indeed, metal coordinated arylcarboxylate complexes (*i.e.*, M-arylcarboxylates) are highly investigated in bio-inorganic chemistry as they show wide structural varieties (with different geometries) and important biological properties [10-12].

"Efficacy enhancement" with "minimal side effects" is always the leading impetus for a medicinal/bioinorganic chemist working in the NSAID area and two approaches that have become highly successful are (a) trying to alter the coordination environment by judicially choosing/replacing a metal ion that is capable of coordinating with a chosen drug, [13-15] (b) using ancillary ligands (*e.g.* triphenylphosphine (tpp), phenanthroline, bipyridyl, pyrazine *etc.,*) that can also penetrate/associate with the existing metal ion coordination environment in the complex [13, 14, 16-18]. It is also possible to use a combination of both the approaches mentioned above. In this regard, copper (mainly in the +2 oxidation state) becomes an important and judicious choice as it is a biologically essential element in most organisms and its absorption, homeostasis and excretion mechanisms are well linked and properly maintained along the evolutionary lines of biological systems [19-21]. Hence, ingestion of copper to living cells, even if through coordinated NSAIDs [22-23] should evoke a minimal problem with regard to side effects of metal poisoning. Moreover, it has been well established that complexation with metal ions can alter the efficacy of a drug [24-26] and it would be rewarding to investigate the copper(II) complexes of an important NSAID, niflumic acid. It is a well-known fact that N-donor compounds, as ancillary ligands, can alter the properties and structures of metal-arylcarboxylates. Nitrogen donor ligands can also enhance the biological activity of transition metal complexes by altering coordination modes of other co-ligands towards the metal center, *e.g.* the cytotoxicity of monomeric $[Cu(asp)_2(py)_2]$ towards various tumor cell lines is higher

than that of the dimeric $[Cu_2(asp)_4]$ complex [27], where py = pyridine. In light of the above discussion we found it pertinent to investigate the spectroscopic, structural and biological activities of the niflumate anion (*nif*) after its complexation with copper(II) in the presence of a variety of N-donor ligands as shown in Scheme 2. The selection of the ancillary ligands, that either belong to a category of flexible aliphatic ligands such as ethylenediamine (*en*), propan-1,3-diamine (*pn*), 1,1-dimethylethylenediamine (*unsym-dmen*)) or that belong to class of resonance-stabilized aromatic ligands such as 3-methylpyridine (*β-pic*), 4-methylpyridine (*γ-pic*), was based on the fact that they can impart structural variations due to different coordination modes of the arycaboxylate group and thereby affect the biological properties.




**Scheme 2:** Ligands used in this work (a) ethylenediamine (*en*), (b) propan-1,3-diamine (*pn*), (c) 1,1-dimethylethylenediamine (*unsym-dmen*), (d) β-picoline (*β-pic*), (e) γ-picoline (*γ-pic*), (f) niflumic acid (H*nif*)

In the literature, a few papers on X-ray structural studies of metal-niflumate complexes; Zn(II) [28], Cu(II) (one monomeric, three dinuclear, one polymeric), [29]] Mn(II) [30] , Co(II) [31], and [Ag(I)] [32] have been reported. Hence, in order to study the coordination chemistry of copper(II) niflumate with aliphatic and aromatic N-donor ligands as an extension of our synthetic methodology [33], we report herein the synthesis and elaborate characterization of five new

copper(II) niflumate complexes, i.e. [Cu(*en*)$_2$(*nif*)$_2$], **1**, [Cu(unsym-*dmen*)(*nif*)$_2$], **2** [Cu(p*n*)$_2$(*nif*)$_2$] [Cu(*pn*)$_2$(H$_2$O)$_2$](*nif*)$_2$, **3**, [Cu(β-pic)$_2$(*nif*)$_2$], **4**, [Cu(γ-pic)$_2$(*nif*)$_2$] **5**, besides their cytotoxic evaluation against various human cancer cell lines for a potential application as anticancer agents. The findings that emerge from these studies can augment the knowledge regarding the synthetic, spectroscopic and structural aspects of Cu(II) arylcarboxylates including NSAIDs, an area to which we have embarked upon for quite some time [34,35]. It is worth mentioning that this work is based upon a methodology concerning the synthesis and characterization of copper(II) arylcarboxylate complexes with N- donor ligands that has been developed over two decades and presents salient features, such as: (i) the materials are cheap and readily available; (ii) the synthetic method is simple, convenient and proceeds without any sophistication at room temperature; (iii) the obtained products are crystalline, pure and with nearly quantitative yield; (iv) usually no recrystallization is needed for further purification; (v) good quality crystals are readily obtained for single crystal X-ray structural analysis.

## 2. Experimental

### *2.1 Materials and methods*

All chemicals and solvents were reagent grade and were used as purchased without any further purification. Elemental analysis was performed using an automatic Perkin Elmer 2400 CHN element analyzer and copper was determined by standard literature methods [36]. Fourier transform infrared spectra (FT-IR) were recorded (neat) on PERKIN ELMER SPECTRUM RX FT-IR system. The UV-vis spectra of the complexes were recorded on a Hitachi U-2001 dual beam spectrophotometer using methanol-water (4:1 v/v) mixture as solvent. The anisotropic X-band (9.43 GHz) EPR spectra of frozen $CHCl_3$ solutions were recorded at 110 K using a Bruker

ESP 300 spectrophotometer. The EPR spectra were referenced to 2,2-diphenyl-1-picrylhydrazyl (DPPH) (g=2.0037). All spectra were recorded using 100 kHz frequency modulation, 21.186 G amplitude modulation, and microwave power of 6 mW.

*2.2 Synthesis of [Cu(en)$_2$(nif)$_2$].2H$_2$O*, **1**

0.50 g $CuSO_4.5H_2O$ (2 mmol) was dissolved in 10 mL of distilled water and sodium niflumate (prepared *in situ* by mixing aqueous sodium hydroxide (0.16 g, 4 mmol) with niflumic acid (1.12 g, 4 mmol)) which was also dissolved in minimum amount of water. On mixing the two solutions, a light green precipitate of hydrated copper(II) niflumate resulted immediately which was filtered through a fine filter paper, washed with water followed by methanol and dried at room temperature (yield 90%). 0.5 g hydrated copper(II) niflumate was suspended in 25mL methanol-water (4:1 v/v) and ethylenediamine (*en*) was added slowly with stirring until a clear violet solution appeared. When the reaction mixture was allowed to evaporate slowly at room temperature, violet crystals appeared after a few days, which were separated from the mother liquor, washed with water and dried in air (yield 90%). Complex **1** is soluble in methanol, acetone but insoluble in water and decomposes at 190 $^oC$. FT-IR (neat, $cm^{-1}$): 3420(m), 3256(m), 3157(s), 2950(m), 1574(s), 1489(m), 1367(s), 1309(s), 1251(m), 1032(s), 941(w), 784(s), 694(s), 541(s); $\Delta\nu_{COO} = \nu_{asym(COO)} - \nu_{sym(COO)} = 207\ cm^{-1}$ · UV-Vis (methanol-water), $\lambda_{max}$(nm) (ε, in $M^{-1}cm^{-1}$): .575 (58). Anal. Cal. for $C_{30}H_{36}N_8O_6F_6Cu$ (MW = 782.21): C, 46.02; H, 4.60, N, 14.32; Cu, 8.18 %; found: C, 46.55; H, 4.32; N, 13.98; Cu, 8.37 %.*2.3 Synthesis of [Cu(unsym-dmen)$_2$(nif)$_2$],* **2**

Complex **2** was prepared in the similar manner as complex **1** by using 1,1-dimethylethylenediamine (*unsym-dmen*) instead of ethylenediamine (*en*). When the reaction mixture was allowed to evaporate slowly at room temperature, violet coloured crystals appeared

after a few days, which were separated from the mother liquor washed with methanol and dried in air (yield 85%). Complex **2** is also partially soluble in water, insoluble in methanol, acetone and decomposes at 201 $^{o}$C. FT-IR (neat, $cm^{-1}$): 3222(s), 3063(m), 2890(m), 2820(w), 1578(s), 1384(s), 1323(s), 1254(m), 1114(m), 899(w), 762(s), 521(m) ; $\Delta\nu_{COO} = \nu_{asym(COO)} - \nu_{sym(COO)}$ = 194 $cm^{-1}$ . UV-Vis (methanol-water), $\lambda_{max}$(nm) (ε, in $M^{-1}cm^{-1}$): 560 (162). Anal. Cal. for $C_{34}H_{40}N_8O_4F_6Cu$ (MW = 801.50): C, 50.90; H, 4.99, N, 13.97; Cu, 7.92 %; found: C, 51.06; H, 5.13; N, 13.68; Cu, 7.82 %.

*2.4 Synthesis of $[Cu(pn)_2(nif)_2].[Cu(pn)_2(H_2O)_2](nif)_2$*, **3**

Complex **3** was synthesized in a similar manner as complex **1** by adding propane-1,3-diamine (*pn*) instead of ethylenediamine (*en*) till a clear blue color solution was obtained. When the reaction mixture was allowed to evaporate slowly at room temperature, blue crystals appeared after four days, which were separated from the mother liquor washed with methanol and dried in air (yield 88%). Complex **3** is also soluble in water, insoluble in methanol and decomposes at 182 $^{o}$C. FT-IR (neat, $cm^{-1}$): 3480(b), 3277(s), 2938(m), 1579(s), 1383(s), 1116 (s), 1202(s), 941(s), 775(s), 670(m), 521(m) 456(w); $\Delta\nu_{COO} = \nu_{asym(COO)} - \nu_{sym(COO)}$ = 197 $cm^{-1}$ . UV-Vis (methanol-water), $\lambda_{max}$(nm) (ε, in $M^{-1}cm^{-1}$): 563 (188). Anal. Cal. for ($C_{64}H_{76}N_{16}O_{10}F_{12}Cu_2$) (MW = 1584.48): C, 48.47; H, 4.79, N, 14.13; Cu, 8.08 %; found: C, 48.55; H, 4.58; N, 14.45; Cu, 8.23 %.

*2.5 Synthesis of $[Cu(\beta\text{-}pic)_2(nif)_2]$*, **4**

Complex **4** was synthesized in a similar manner as complex **1** by adding β-picoline (*β-pic*) in place of ethylenediamine (*en*) until a clear blue color solution was obtained. When the reaction mixture solution was allowed to evaporate slowly at room temperature, blue crystals appeared after three days, which were separated from the mother liquor, washed with water and dried in

air (yield 85%). Complex **4** is insoluble in water and soluble in methanol and decomposes at 183 $^{o}$C. FT-IR (neat, $cm^{-1}$): 3260(m), 2980(m), 1587(s), 1518(s), 1443(m), 1336(s), 1065(s), 775(s), 660(s), 534(s), 458(m); $\Delta\nu_{COO} = \nu_{asym(COO).} - \nu_{sym(COO)} = 251\ cm^{-1}$ · UV-Vis (methanol-water), $\lambda_{max}$(nm) (ε, in $M^{-1}cm^{-1}$): 728 (108). Anal. Cal. for $C_{38}H_{30}N_6O_4F_6Cu$ (MW = 812.22): C, 56.14; H, 3.69, N, 10.34; Cu, 7.87 %; found: C, 56.33; H, 3.55; N, 10.26; Cu, 7.53 %.

*2.6 Synthesis of [Cu(γ-pic)$_2$(nif)$_2$],* **5**

Complex **5** was synthesized in a similar manner as complex **1** by adding *γ*-picoline (*γ-pic*) in place of ethylenediamine until a clear royal blue color solution was obtained. When the reaction mixture solution was allowed to evaporate slowly at room temperature, royal blue crystals appeared after a few days, which were separated from the mother liquor washed with water and dried in air (yield 92%). Complex **5** is also insoluble in water and soluble in methanol, other organic solvents and decomposes at 210 $^{o}$C. FT-IR (neat, $cm^{-1}$): 3069(s), 1595(s), 1346(s), 1445(3), 1066(m), 761(s), 547(m), 492(s); $\Delta\nu_{COO} = \nu_{asym(COO)} - \nu_{sym(COO)} = 222\ cm^{-1}$ · UV-Vis (methanol-water), $\lambda_{max}$(nm) (ε, in $M^{-1}cm^{-1}$): 735 (137). Anal. Cal. for $C_{38}H_{30}N_6O_4F_6Cu$ (MW = 812.22): C, 56.14; H, 3.69, N, 10.34; Cu, 7.87 %; found: C, 55.97; H, 3.76; N, 10.17; Cu, 7.93 %.

*2.7 Biological tests*

*Cell Cultures*. Two cell types were studied. The HT-144 and SKMEL-28 cell lines derived from human melanoma and were obtained from the American Tissue Culture Collection (ATCC). They were cultured in DMEM, Mc Coy's 5a modified or MEM culture medium (pH 7.4, controlled by the presence of the pH indicator (phenol red) in the medium supplemented with 10 % FBS.

*Cell viability assay.* Initial stock solutions of complexes were prepared in dimethyl sulfoxide (DMSO) at a concentration of $10^{-1}$ M and used to achieve the several dilutions. The effects of complexes on cell viability at 24h were measured using an MTT tetrazolium salt colorimetric assay (Sigma-Aldrich, Saint-Louis, Missouri, United States) according to the manufacturer's instructions. Briefly, the cells (15000/well) were incubated in three replicates in a 96-well plate in the presence of various concentrations of complexes (0, 6.25, 12.5, 25, 50, 100, 200 µM in the wells). After 24 hours of treatments, cells were incubated for three hours after addition of 20% MTT, then medium was removed and DMSO was added to dissolve formazan. A colorimetric assay was realized at 560nm using a plate reader Tecan F200 Pro (Tecan, Lyon, France). The 50% inhibitory concentration (IC50) for each complex was defined as the concentration producing 50% decrease in cell growth.

*2.8 Statistical Analysis*

The data were expressed as the mean _ SD of 3 independent experiments. Each experiment was performed in triplicate. The significance of differences was established with the Student's t-test.

*2.9 X-ray Crystallography*

Single-crystal diffraction data for complexes **1**, **3** and **5** were collected at 295 K on a Nonius Kappa diffractometer equipped with a CCD detector with graphite-monochromated Mo-Kα radiation (λ = 0.71069 Å ). Intensities were corrected for Lorentz, polarization and absorption effects [37]. The low quality/smallness of the crystals of **1** and **5** did not allow a high data completeness fulfilment. The structures were solved by direct methods with the SIR97 program [38] and refinements were performed on $F^2$ by full matrix least-squares methods with all non-H atoms anisotropic, apart from the disordered O1w atom in **3** which was refined isotropically. In complex **5**, the fluorine atoms of the $CF_3$ group of the niflumic ligand were found to be

disordered over two equivalent positions. In all structures, the C-H hydrogens were included on calculated positions, riding on their carrier atoms. All other N/O-H hydrogen atoms were located in the difference-Fourier map and refined isotropically, apart from those of the water molecule coordinated to Cu2 in complex **3** (not included in the refinement).

Diffraction data for complex **4** were collected as well; in spite of many efforts, however, it was not possible to refine the structure to a reasonable R factor value. For this reason the geometry of the complex was optimized *via* DFT calculations (vide infra). All calculations were performed using SHELXL2014/7 [39] implemented in the WinGX system of programs [40]. Experimental details are given in Table S1 (Supplementary data).

Crystallographic data for the structural analysis of the three new compounds have been deposited at the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, UK, and are available free of charge from the Director on request quoting the deposition number CCDC 1824661-1824663 for complexes **1, 5** and **3** respectively.

*2.10 DFT calculations*

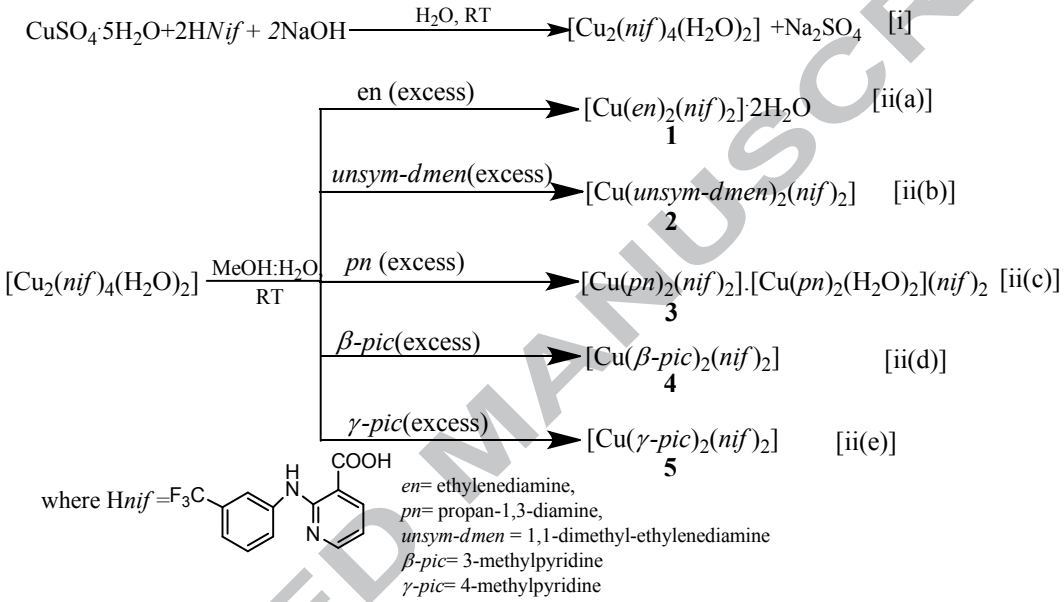
The geometry of the complex $[Cu(\beta\text{-pic})_2(nif)_2]$ **4** was optimized *in vacuum* without any symmetry constraints, starting from the crystal structure geometry. Calculations were carried out with the Gaussian09 suite of programs [41] employing the B3LYP [42] functional in combination with a LANL2DZ [43] basis set.

## 3. Results and Discussion

*3.1 Synthesis*

The hydrated copper(II) niflumate was obtained by reacting copper sulfate pentahydrate with the sodium salt of niflumic acid as shown in Scheme 3 (eq. (i)). The precipitated product

was then suspended in methanol-water mixture (4:1, v/v) followed by the addition of different nitrogen-donor ligands (*en*, *pn*, *unsym-dmen*, *β-picoline* and *γ-picoline*) with continuous stirring until a clear blue/violet solution was observed in each case. Upon slow evaporation of the respective resultant reaction mixtures at room temperature, five new copper(II) niflumate complexes **1-5** were isolated in good yields.




**Scheme 3.** Schematic representation of the synthesis of complexes **1-5**

The composition of each complex has been confirmed by elemental analyses and the exact structure of complexes **1, 3** and **5** was confirmed by X-ray structure determinations.

*3.2 X-Ray crystal structures of complexes **1, 3** and **5***

The ORTEPIII views [44] of complexes **1**, **3** and **5** are shown in Figs. 1, 2 and 3, respectively. In all structures, the central copper atom lies on a symmetry centre. In complex **1**, the coordination geometry is elongated octahedral, the Cu atom being coordinated to two chelating *en* ligands and two monodentate niflumate ligands in apical positions. For each complex, outside the first coordination sphere, there are two co-crystallized water molecules. The five membered ring Cu1-

N1-C1-C2-N2 adopts a twisted conformation according to the Cremer and Pople analysis (puckering amplitude $q_2$ = 0.44, pseudorotation phase angle $\varphi_2$ = 122.9°) [45]. The Cu-N distances, reported in Table 1, are perfectly in line with those found in Cu(en) structures, for which the mean Cu-N distance is 2.01(3) Å (571 structures in CSD).

The structure of complex **3** is characterized by the presence of two different Cu complexes, as shown in Fig. 2. In the first one (Cu1 complex) the coordination is similar to that found in complex **1**, with the niflumate ligands in apical positions of a distorted octahedron; conversely, the second copper atom (Cu2) is bound with a distorted octahedral geometry to two *pn* chelating ligands and two water molecules to give a cationic species, while the niflumate anionic moieties are located outside the first coordination sphere. Both the Cu1-N1-C1-C2-C3-N2 and Cu2-N5-C17-C18-C19-N6 six-membered rings adopt a chair conformation, with a total puckering amplitude of 0.609 and 0.672 and spherical polar angles of 7.42 ° and 9.13°, respectively.

**Table 1.** Selected bond distances and angles (Å, °) for complexes **1, 3** and **5.**

Complex **1**

| | | | |
|---|---|---|---|
| Cu1 - O1 | 2.606(2) | Cu1 - N1 | 2.008(2) |
| Cu1 - N2 | 2.010(2) | | |
| | | | |
| O1 - Cu1 - N1 | 91.97(7) | N1 - Cu1 - N2 | 84.66(9) |
| O1 - Cu1 - N2 | 88.09(7) | N1 - Cu1 - N2$^{i}$ | 95.34(9) |

Complex **3**

| | | | |
|---|---|---|---|
| Cu1 - N1 | 2.018(2) | Cu1- N2 | 2.011(3) |
| Cu1 - O1 | 2.685(2) | Cu2- N5 | 2.016(2) |
| Cu2- N6 | 2.011(2) | Cu2- O1W | 2.500(8) |
| | | | |
| N1 - Cu1 - N2 | 89.6(1) | N1 - Cu1 - N2$^{ii}$ | 90.3(1) |
| O1 - Cu1- N2 | 101.8(1) | O1 - Cu1- N1$^{ii}$ | 86.0(1) |
| O1 - Cu1- N1 | 94.0(1) | O1 - Cu1 - N2$^{ii}$ | 78.2(1) |
| N5 - Cu2 - N6 | 86.6(1) | N5 - Cu2 - O1W | 85.4(2) |
| N6 - Cu2 - O1W | 89.6(2) | N5 - Cu2 - N6$^{iii}$ | 93.3(1) |
| O1w - Cu2 - N6$^{iii}$ | 90.4(2) | O1w - Cu2 - N5$^{iii}$ | 94.6(1) |

Complex **5**

| | | | |
|---|---|---|---|
| Cu1 - N1 | 2.051(2) | Cu1 - O2 | 2.664(2) |
| Cu1 - O1 | 1.942(2) | | |
| | | | |
| N1 - Cu1 - O1 | 90.21(8) | O1- Cu1- O2 | 54.51(8) |
| N1 - Cu1 - O2 | 89.11(8) | O1- Cu1- O2$^{iv}$ | 125.49(8) |

Equivalent positions: (i) 1-x,-y,1-z; (ii) 1-x,1-y,1-z; (iii) -x,1-y,1-z; (iv) -x,-y,1-z

**Table 2.** Structural parameters of hydrogen bonds (Å, °) for complexes **1, 3** and **5.**

| **D-H…A** | **D-H** | **D…A** | **H…A** | **D-H…A** |
|---|---|---|---|---|
| Complex **1** | | | | |
| N1-H ...O2 | 0.84(3) | 3.053(3) | 2.26(3) | 158(2) |
| N4-H ...O2 | 0.89(3) | 2.672(3) | 1.92(3) | 141(2) |
| O1W-H...O2 | 0.76(4) | 2.799(3) | 2.05(4) | 172(4) |
| N2-H ...O1W$^{i}$ | 0.90(3) | 3.042(3) | 2.24(3) | 148(3) |
| C12-H...F3$^{ii}$ | 0.93 | 3.507(3) | 2.62 | 157 |
| N2-H ...O1W$^{iii}$ | 0.79(4) | 3.121(3) | 2.33(3) | 173(3) |
| N1-H ...O1W$^{iv}$ | 0.93(3) | 3.093(3) | 2.26(3) | 147(2) |
| O1W-H...O1$^{v}$ | 0.78(4) | 2.828(3) | 2.06(4) | 175(4) |

Equivalent positions: (i) 3/2-x,y-1/2,z; ( ii) x+1/2,y,3/2-z; (iii) 1-x,-y,1-z; (iv) x-1/2,1/2-y,1-z; (v) 2-x,-y,1-z

| | | | | |
|---|---|---|---|---|
| Complex **3** | | | | |
| N3-H...O1 | 0.70(3) | 2.656(3) | 2.03(3) | 150(3) |
| N7-H...O3 | 0.76(3) | 2.660(3) | 1.99(3) | 149(3) |
| C3-H...N4$^{i}$ | 0.97 | 3.386(5) | 2.50 | 151 |
| N5-H...O2$^{ii}$ | 0.85(4) | 2.930(3) | 2.09(4) | 166(3) |
| N1-H...O4$^{iii}$ | 0.91(4) | 3.081(3) | 2.21(4) | 161(4) |
| N1-H...O2$^{iv}$ | 0.90(4) | 2.894(4) | 2.07(4) | 150(3) |
| N6-H...O2$^{iv}$ | 0.85(4) | 2.887(3) | 2.05(4) | 167(4) |
| N2-H...O4$^{v}$ | 0.85(5) | 3.032(4) | 2.19(5) | 172(5) |
| N5-H...O4$^{vi}$ | 0.76(4) | 2.964(4) | 2.24(4) | 167(4) |
| N6-H...O3$^{vi}$ | 0.96(4) | 3.005(3) | 2.05(4) | 163(3) |

Equivalent positions: (i) x,1/2-y,z+1/2; (ii) x-1,y,z; (iii) x,1/2-y,z-1/2; (iv) 1-x,1-y,-z-1; (v) 1-x,y+1/2,-z-1/2; (vi) -x,y+1/2,-z-1/2

| | | | | |
|---|---|---|---|---|
| Complex **5** | | | | |
| N3-H ...O2 | 0.82(3) | 2.646(2) | 1.93(3) | 144(3) |

In complex **5** (Fig. 3) the Cu atom is bound to two niflumate and two $\gamma$-picoline ligands; the niflumate acts as a bidentate ligand coordinated *via* the carboxylate group, with very different Cu-O distances (Table 2), one much longer than the other. The resulting geometry is highly distorted octahedral.

It is worth mentioning that in all the three structures there is the formation of a quite strong charge-assisted intramolecular N-H…O hydrogen bond (Table 2) involving the NH and carboxylate groups of the niflumate moiety, the only difference consisting in the fact that in **1** this intramolecular interaction involves the non-coordinated niflumate oxygen atom.

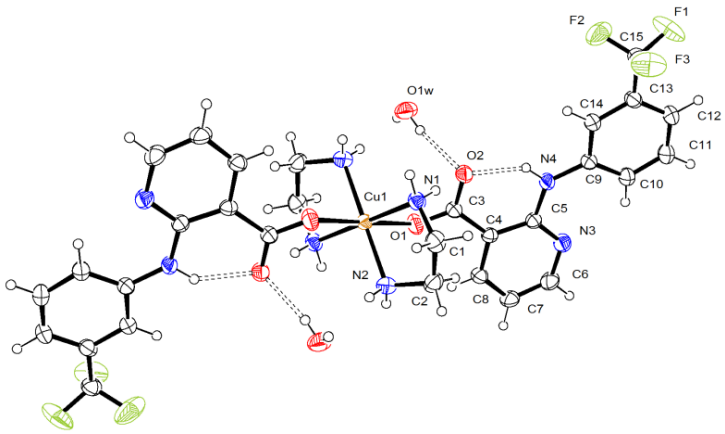



**Fig. 1**. ORTEPIII view and atom numbering scheme for complex **1** $[Cu(en)_2(nif)_2].2H_2O$. Thermal ellipsoids are drawn at the 40% probability level. Hydrogen bonds are drawn as dashed lines.

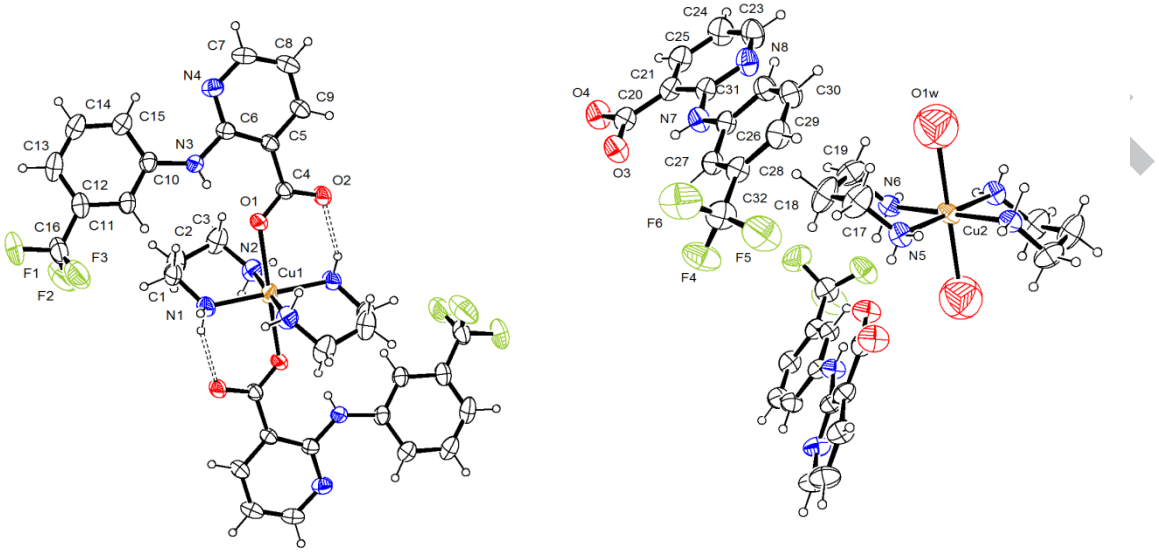



**Fig. 2**. ORTEPIII view and atom numbering scheme for complex **3:** $[Cu(pn)_2(nif)_2]$. $[Cu(pn)_2(H_2O)_2](nif)_2$ showing the two different Cu coordinations (arbitrary projections). Thermal ellipsoids are drawn at the 40% probability level. Hydrogen bonds are drawn as dashed lines. Intramolecular hydrogen bonds are not shown for clarity.

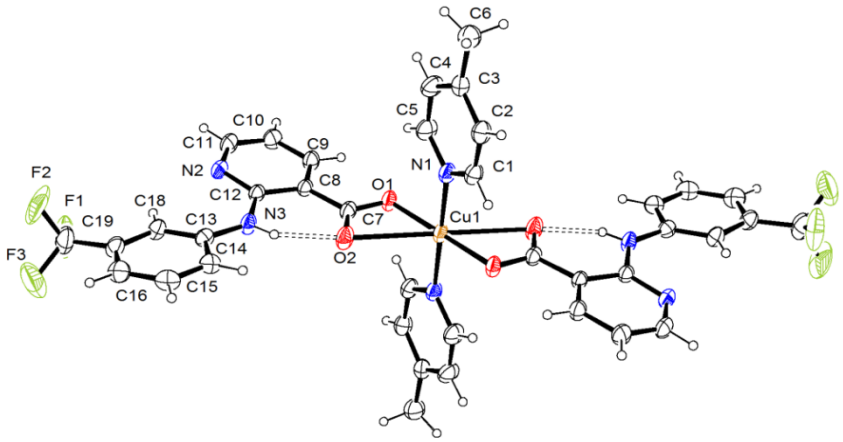



**Fig. 3**. ORTEPIII view and atom numbering scheme for complex **5** [Cu(*γ*-picoline)$_2$(*nif*)$_2$]. Thermal ellipsoids are drawn at the 40% probability level. Hydrogen bonds are drawn as dashed lines. Only one position of the disordered $CF_3$ group is shown for clarity.

Since it was not possible to obtain a good refined structure of the [Cu($\beta$-picoline)$_2$(*nif*)$_2$] complex **4**, its geometry has been obtained via DFT calculations and it is shown in Fig. 4. Here, some relevant bond distances are also reported. The geometry is very similar to that of the related complex **5**, including the presence of the strong intramolecular N-H…O hydrogen bond (N…O distance= 2.658 Å).

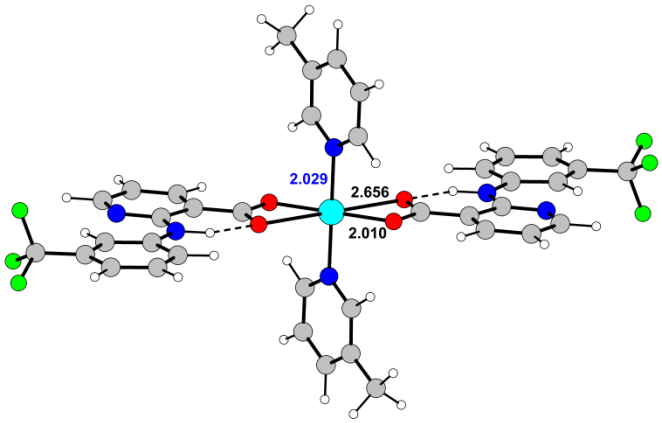



**Fig. 4**. DFT optimized geometry of complex **4** [Cu($\beta$-picoline)$_2$(nif)$_2$]. Hydrogen bonds are drawn as broken lines; Cu-N in blue.

In **1**, due to the abundance of good donors and acceptors, the packing diagram is mainly determined by the formation of O/N-H…O hydrogen bonds. The projection of the unit cell along *a* (Fig. 5) clearly shows the formation of a Cu polyhedra/organic layered structure; the cocrystallized water molecules have the task of joining adjacent complexes, acting both as hydrogen bond donors towards the oxygens of the carboxylate group, with the formation of a R4,4(12) ring (Fig.5b), and as acceptors for the $NH_2$ groups. In **3**, the hydrogen bonding network is even more complicated. In addition to the long list of intermolecular interactions reported in Table 2, a short contact O1W...O3( x,1/2-y,z-1/2) of 2.682(8) Å is indicative of an hydrogen bond involving the coordinated water molecule. Overall, the packing diagram is made of rows of

alternating Cu1/Cu2 complexes as shown in Fig. 6; two adjacent Cu1/Cu2 different complexes are linked to each other through N-H…O hydrogen bonding interactions involving O2 as acceptor and N5 and N6 as donors (Table 2).

As for complex **5**, no intermolecular hydrogen bond of some importance was observed, due to the lack of good donor and acceptor groups.

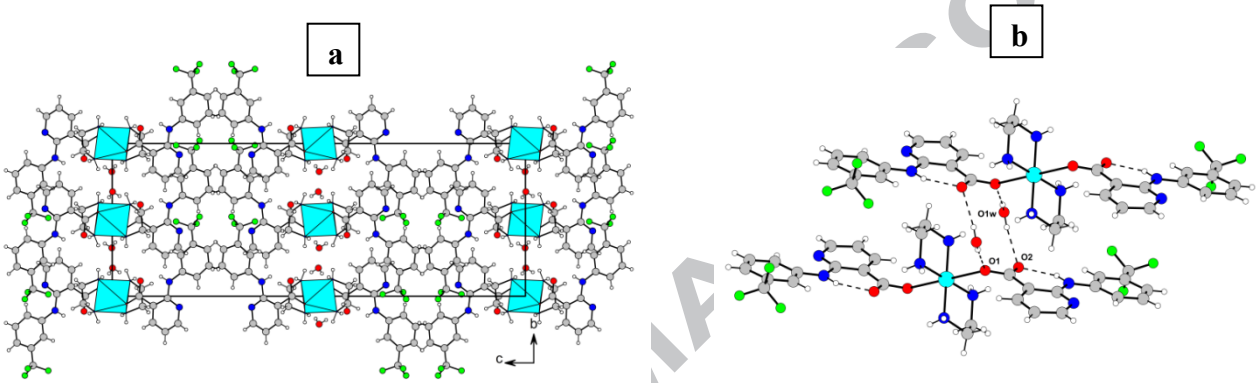



**Fig. 5**. (a) Unit cell content of **1** (viewed along the a axis); (b) R4,4(12) ring. Hydrogen bonds are drawn as broken lines.

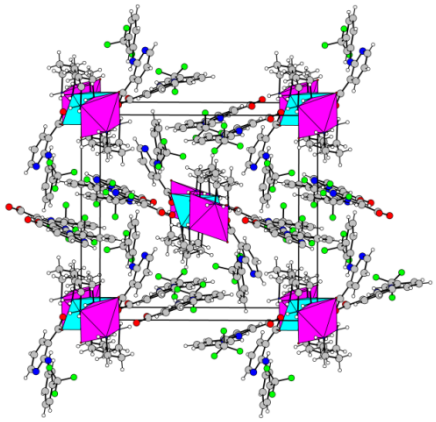


**Fig. 6**. Unit cell content of **3**. Polyhedra around the Cu1 and Cu2 atoms are shown in blue and magenta, respectively.

*3.3 FT-IR and electronic spectroscopy*

Infrared spectra of complexes **1-5** were recorded in the region 4000-400 $cm^{-1}$ and tentative band assignments have been made on the basis of earlier reports in the literature [46, 47]. In the solid state FT-IR spectra of complexes **1-5**, significant broad peaks are observed in the region 3500-31000 $cm^{-1}$, indicating the O-H and N-H stretching frequency of water and niflumate or nitrogen donor ligands (such as *en*, *pn*, *usym-dmen*) in these complexes. The absorption bands observed in the region 3100-2900 $cm^{-1}$ were assigned to C($sp^2$)-H stretching vibrations of the niflumate anion in all complexes. The absorption peaks in the region 1650-1600 $cm^{-1}$ were assigned to C=C stretching vibrations of the niflumate molecule. The sharp bands in the region 1600-1500 $cm^{-1}$ and 1400-1340 $cm^{-1}$ correspond to the $\nu_{as(COO)}$ and $\nu_{s(COO)}$ stretching vibrations of the carboxylate group of niflumate [8, 13, 46, 47]. The parameter $\Delta\nu$ ($\nu_{as(COO)}$-$\nu_{s(COO)}$) can be used as an important tool in assigning the mode of coordination of the carboxylate ligand in metal-carboxylate complexes. From various coordination modes of carboxylate coordination, i) ionic, ii) unidentate, iii) bidentate chelating are the most common. In complexes **1-3**, the $\Delta\nu_{(COO)}$ values of 207, 194, 175 $cm^{-1}$ , respectively, fall in the range (210-160 $cm^{-1}$) observed for various ionic or monodentate complexes e.g. sodium formate ($\Delta\nu$=201$cm^{-1}$), sodium acetate ($\Delta\nu$=164 $cm^{-1}$), sodium ibuprofenate ($\Delta\nu$=190 $cm^{-1}$), sodium diclofenate ($\Delta\nu$=170) [48]. *etc.* For complexes **4** and **5**, the higher value of $\Delta\nu$ = 251, 222 $cm^{-1}$ , respectively, indicates a bidentate chelation mode of the carboxylate anion ~~representing covalent nature of these complexes~~. The absorption peaks observed in the region 1000-620 $cm^{-1}$ might be assigned to in-plane bending and out-of-plane deformation vibrations of hydrogen atoms on aromatic rings. The absorption peaks observed around 500 $cm^{-1}$ in complexes **1-5** are within the range reported for Cu-O and Cu-N

stretching frequencies in the literature. The FT-infrared spectra for complexes **1-5** are shown in Fig.S1 (supplementary data).

The UV-Visible spectra of complexes **1-5** were recorded using methanol-water (4:1) as solvent and exhibit very broad d-d bands in the visible region from 500-800 nm, corresponding to an octahedral complex or a combined merged broad peak corresponding to a square pyramidal or square planar geometry. So, the exact geometry of the complex could not be predicted from solution state UV-Vis spectra. UV-Vis spectra ~~of complexes 1-5~~ showed a broad absorption band at 575 nm ($\varepsilon$= 58 $L.mol^{-1}.cm^{-1}$), 560 ($\varepsilon$= 162 $L.mol^{-1}.cm^{-1}$), 563 ($\varepsilon$= 188 $L.mol^{-1}.cm^{-1}$), 728 ($\varepsilon$= 108 $L.mol^{-1}.cm^{-1}$) nm 735 ($\varepsilon$= 137 $L.mol^{-1}.cm^{-1}$) nm for complexes **1-5,** respectively, indicating that complexes **1-3** have a similar chromophore and complexes **4** and **5** have different chromophore. The ~~observed~~ UV-vis of complex **3** shows only one broad absorption band in the region 570-550 nm indicating the presence of a $CuN_4O_2$ chromophore, so it is not possible to distinguish the two types of coordination environments around the two copper(II) atoms in asymmetric unit as revealed by the X-ray structure determination. The UV-visible spectra of all complexes **1-5** are shown in Fig. S2 (supplementary data). The observed electronic spectral bands for complexes **1-5** are in good agreement with literature data [49]. To investigate charge transfer transitions, UV-visible spectra of micromolar solutions (water-methanol as solvent, 4:1 v/v) of all complexes were recorded in the range 400-200 nm. The observed peaks at around 290 nm for all complexes correspond to a charge transfer transition between the $\pi$ electron cloud of the niflumate moiety and the copper(II) metal ion as shown in Fig. S3 (supplementary data). In order to investigate variations in coordination in solution with respect to the solid state, UV-vis spectra in the solid state of complexes **1-5** have been recorded: they do not show any significant change in absorption maxima in comparison to the solution state, indicating an identical

coordination geometry of the copper(II) complexes in solution state. This is in accordance with a relative stability of the complexes in solution, since no modification in the UV-visible spectra was observed over time. The solid state UV-Vis spectra of complexes **1-5** are shown in Fig.S3 (supplementary data).

*3.4 EPR Spectroscopy*

The EPR spectra of Cu(II) complexes **1-5**, together with the reference complex $Cu(nif)_2.2H_2O$, obtained in $CHCl_3$ solution at 110 K at X-band (9.43 GHz) demonstrate a typical pattern of copper complexes with hyperfine structure resulting from the coupling of the $Cu^{2+}$ electron spin ($S$=1/2) with its nuclear spin ($I$=3/2) (Fig. S5 of the supplementary data). The obtained spin-Hamiltonian parameters ($g_{//}$, $A_{//}$ and $A_{\perp}$, $g_{\perp}$), reported in Table 3, are typical for an axially elongated $d_{x^2-y^2}$ ground state ($g_{||} > g_{\perp} \approx 2.0023$) for $Cu^{2+}$ ions with an octahedral configuration geometry around the central ion. Two complexes "families" can be evidenced according to these parameters. Indeed, Kivelson and Neiman [50] have reported that $g_{||}$ values less than 2.3 indicate considerable covalent character of the metal-ligand bonds, while a value greater than 2.3 indicates an ionic character. As the $g_{||}$ value of complexes **1**, **2**, **3**, and $Cu(nif)_2.2H_2O$ is found to be less than 2.3 it implies a significant covalent character of the metal-ligand bonds in these complexes. This is in line with the monodentate binding mode of the niflumate ligand observed in these cases. On the other hand, in view of the Peisach-Blumberg diagram [51], their obtained values of $g_{//}$ and $A_{//}$ are consistent with a $CuN_4O_2$ chromophore (except for $Cu(nif)_2.2H_2O$). In contrast, in the case of complexes **4** and **5**, including a picoline ligand and where the niflumate ligand is bidentally coordinated (X-ray crystallography section), the $g_{||}$ value higher than 2.3 indicates an ionic character of the $Cu^{2+}$-niflumate bonds. Moreover, following the Peisach-

Blumberg diagram, their spin-Hamiltonian parameters underline a $CuO_4N_2$ chromophore. Finally, the EPR parameters of Cu(*nif*)$_2$.2H$_2$O are typical of a $CuO_4$ chromophore.

**Table 3.** EPR parameters for copper complexes **1-5** and the Cu(nif)$_2$.2H$_2$O reference complex

| Complex | Tensor | $10^{-4}$ cm$^{-1}$ | $10^{-4}$ cm$^{-1}$ |
|---|---|---|---|
| Cu(*nif*)$_2$.2H$_2$O | $g_\perp$ = 2.085<br>$g_{//}$ = 2.271 | $A_\perp$ = 23<br>$A_{//}$ = 181 | $LW_\perp$ = 32<br>$LW_{//}$ = 42 |
| Complex **1** | $g_\perp$ = 2.032<br>$g_{//}$ = 2.229 | $A_\perp$ = 16<br>$A_{//}$ = 196 | $LW_\perp$ = 25<br>$LW_{//}$ = 29 |
| Complex **2** | $g_\perp$ = 2.045<br>$g_{//}$ = 2.242 | $A_\perp$ = 18<br>$A_{//}$ = 191 | $LW_\perp$ = 28<br>$LW_{//}$ = 45 |
| Complex **3** | $g_\perp$ = 2.035<br>$g_{//}$ = 2.251 | $A_\perp$ = 19<br>$A_{//}$ = 187 | $LW_\perp$ = 30<br>$LW_{//}$ = 38 |
| Complex **4** | $g_\perp$ = 2.040<br>$g_{//}$ =2.321 | $A_\perp$ =25<br>$A_{//}$ = 147 | $LW_\perp$ = 47<br>$LW_{//}$ = 39 |
| Complex **5** | $g_\perp$ = 2.042<br>$g_{//}$ =2.326 | $A_\perp$ =26<br>$A_{//}$ = 149 | $LW_\perp$ = 51<br>$LW_{//}$ = 40 |

*3.5 Biological activity*

*In vitro* assays using mammalian cell cultures provide valuable information about the toxicity of compounds and can be used as an accurate approach to estimate acute toxicity. The basal cytotoxic effects of the copper complexes were tested by a colorimetric assay on 2 human melanoma cell lines HT-144 and SKMel -28, which is a first step for further toxicity studies. Cell viability and IC50 values of complexes in HT-144 and SKMel -28 cells are reported in Figure 7 and Table 4. All Cu-niflumate complexes showed higher cytotoxicity than the parent molecule (IC50 > 500 µM, data not shown) against the two human cell cultures. While [$Cu_2$(nif)$_4$.2H$_2$O], complexes **1** and **2** exhibit a similar activity towards the two tumoral cell lines with low

cytotoxicity ($IC_{50}$ higher than 120 μM), comparison of the cytotoxic activities of complexes **3-5** indicates a differential cytoxycity toward the two cell lines. Each complex shows a significant cytotoxicity on the human melanoma cell line HT-144 (with a mean $IC_{50}$ of 69 μM for complex **3**, 50 μM for complex **4** and 33 μM for complex **5**) whereas weak cytotoxic activities of the complexes are observed on the human melanoma cell line SKMel-28 with a mean $IC_{50}$ higher than 130 μM for the 3 complexes. Previous work demonstrated that similar Cu(II)-Ibuprofen complexes induce significant cytotoxicity on the HT-144 cell line whereas a weak cytotoxic activity of the complexes is observed on the healthy human dermal fibroblast HDF cells, and the human melanoma cell line SKMel-28, too [33]. It is interesting to note that in the case of complexes **4** and **5**, showing the highest cytotoxicity, the niflumate ligand is bidentate whereas in the case of the other complexes it is monodentate; moreover, as mentioned above, in complex **1**, at variance with the other cases, the intramolecular NH…O hydrogen bond involves the non-coordinated niflumate oxygen. These structural characteristics could explain the cytotoxicity differences.

**Table 4.** $IC_{50}$ of complexes in HT-144 and SKMel-28 cells at 24h as determine by a colorimetric assay. Data are expressed as $IC_{50}$ values (µM) and are means ± SD of 3 independent experiments.

| Complexes | $IC_{50}$ (µM) | |
|---|---|---|
| | HT-144 | SKMel-28 |
| Cu(nif)$_2$.2H$_2$O (**S0**) | 141 ± 8 | 158 ± 7 |
| Complex **1** (**S1**) | 124 ± 13 | 162 ± 8 |
| Complex **2** (**S2**) | 159 ± 6 | 163 ± 7 |
| Complex **3** (**S3**) | 69 ± 9 | 147 ± 9 |
| Complex **4** (**S4**) | 50 ± 8 | 137 ± 9 |
| Complex **5** (**S5**) | 34 ± 8 | 134 ± 9 |
| Cu(Ibu)$_2$.xH$_2$O [33b] | 113 ± 23 | > 200 |

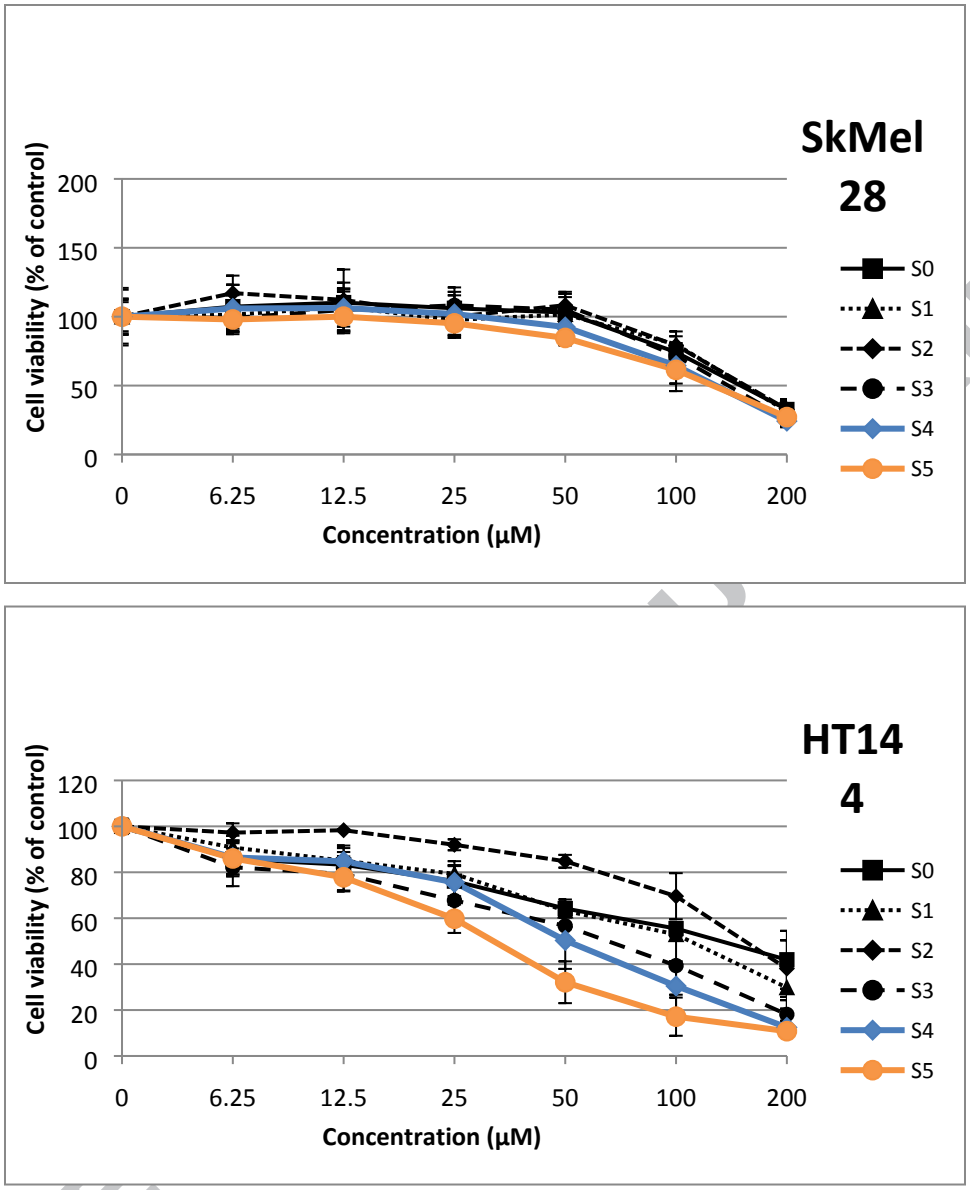



**Fig. 7.** Cytotoxic effects of the complexes on tumoral (human dermal melanoma cell lines HT-144 and SKMEI-28) human cell lines. Each cell line was treated (0-200 µM) with Cu(II) complexes for 24h and viable cells were determined using a colorimetric assay. Each concentration point represents the mean ± S.E.M. of triplicate samples.

## 4. Conclusions

Five new copper(II)-niflumate complexes **1-5** have been synthesized in the presence of different nitrogen-donor ligands using methanol-water (4:1 v/v) as solvent in nearly quantitative yield at room temperature. The newly synthesized copper(II) complexes were characterized by elemental analyses and spectroscopic methods (UV-Vis, FT-IR, EPR). The structures of complexes **1**, **3** and **5** have been unambiguously determined by X-ray structure determination, confirming the formation of mononuclear covalent complexes. The structure of complex **4** has been optimized with the help of DFT calculations which provided insight into the possible existence of a structure similar to that of complex **5**. The EPR spectra revealed a typical pattern of copper complexes with hyperfine structure resulting from the coupling of the $Cu^{2+}$ electron spin ($S$=1/2) with its nuclear spin ($I$=3/2). The $g_{\|} < 2.3$ for complexes **1-3** imply covalent character which is in line with the monodentate coordination mode of the anionic niflumate ligand, whereas the values of $g_{//}$ and $A_{//}$ for complex **4** and **5** is consistent with a $CuN_4O_2$ chromophore. Complexes in which the niflumate ligand is bidentate (**4** and **5)** showed significantly higher cytotoxicity against the human melanoma cell line HT-144 than the other complexes (**1-3)**, where niflumate exhibits a monodentate coordination mode.

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**Supplementary Data**

Supplementary data content: details of the IR, UV-Vis and EPR spectra (5 figures) and X-ray crystallography (1 table).

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**Highlights**

- Five new copper(II)-niflumate complexes have been synthesized and characterized
- Their structures have been determined by X-ray diffraction and DFT calculations
- Cytotoxic effects of the complexes on tumoral human cell lines have been evaluated
- The niflumate coordination mode affects cytotoxicity against the cell line HT-144

**Synopsis**

New copper(II) complexes containing the non-steroidal anti-inflammatory drug niflumic acid as ligand have been synthesized and characterized. Cytotoxic effects of the complexes on tumoral human cell lines have also been evaluated.

**Graphycal abstract**