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Zn(II) and Cu(II) complexes containing bioactive O,O-chelated ligands: homoleptic and heteroleptic metal-based biomolecules†

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Historically, many useful drugs have been developed from natural sources and their mechanisms of action deeply investigated for therapeutic applications. Recently, the interaction between pharmacologically active biomolecules and transition metal ions has open the way towards the construction of new drugs, where the unique properties of metal complexes are joint with the specific mechanisms of action of the coordinated bio ligands of natural extraction. In this context, this perspective summarizes some recent research work devoted to the develop of new metal-based drugs containing Zn(II) or Cu(II) metal ions. Both metals have a strong tendency to form highly stable complexes with N,N- and O,O-donor ligands bounded through chelation, giving rise, particularly when the bounded organic molecules are drug candidates of natural extraction, to delivery drug systems, new biologically active complexes and potential diagnostic agents due to their intrinsic spectroscopic properties.

Introduction

Over the last decade medicinal inorganic chemistry has been the scene of a developing area of pharmaceutical research, based on massive investigation into new anticancer and antimicrobial metal-based therapeutic agents. A rational drug design has focused on a number of transition metal complexes other than platinum, within the field of cancer treatment, offering unique properties such as variable redox states, photophysical properties, possibility of tailoring substrate specificity and ability of targeting DNA through non covalent modes of action.[1-7] The metallo-drug field is interestingly evolving towards compounds preferably targeting DNA through non covalent modes of action such as groove binding, phosphate clamps, insertion or intercalation. In particular, the intercalation mode induces the most dramatic changes in the DNA structure. Molecules with extended planar aromatic systems can insert between paired DNA bases through π–π stacking and thus affording significant unwinding, stiffening and lengthening of the helix.[8,9] This is why intercalators can overcome resistance phenomena common to other drugs. Current metallo-intercalators are typically either coordinatively saturated square planar Pt(II) or octahedral Rh(III) and Ru(II) complexes based on aromatic nitrogen ligands.[10] These compounds are extensively used as structural probes for DNA or as diagnostic agents rather than as anticancer agents.[2]

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In this context, the development of new metal-based drugs with structures suitable for the intercalation mode of action, containing metals such as zinc and copper has been less explored. Despite the variety of physiological roles of the Zn(II) ion[11-13] and the wide repertoire of Zn(II) complexes utilized in many fields (binder complexes at DNA sites,[14-17] radioprotective agents,[18] tumor photosensiters,[19] antidiabetic insulin-mimetic[20,21] and antibacterial or antimicrobic activities[22-24]) only very recently a large interest is growing in the use of Zn(II) coordination complexes with low toxicity and low side effects in medicinal therapeutic applications,[25] for treating diabetes mellitus[26] or cancer.[27-34]

Differently than zinc, copper, an essential microelement found in all living organisms, shows the unique ability to adopt two different oxidation states (Cu(II) and Cu(I)), giving rise, in metal complexes, to versatile coordination states and geometries. Due to the permeability of cancer cell membranes to copper compounds, a number of copper complexes have been screened for their anticancer activity and some of them found active both in vivo and in vitro.[35,36] An excellent review has just appeared in literature, summarizing that the great variety of copper complexes used for therapy or diagnosis of various diseases, are mostly monomeric and dimeric molecules, primarily containing ligands with N and O donating atoms and in a square planar arrangement.[37] Both zinc and copper ions have a strong tendency to bound through chelation N,N- and O,O-donor ligands, giving rise, particularly when the bounded organic molecules are drug candidates of natural extraction,[38,39]

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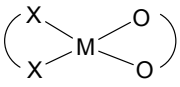
to both delivery drug systems and new biologically active complexes.[40]

Considering the fact that significant progresses have been made during the last few years in this field, in this perspective we would like to summarize the recent developments in the construction of Zn(II) and Cu(II) complexes, containing a sufficient number of aromatic planar fragments to be potential intercalating metal agents, specifically built up through the choice of O,O chelating ligands, bioactivity and of natural extraction. We will give examples of Zn(II) and Cu(II) O,O-chelated complexes where the coordination sphere is completed by both the same ligands, with the generation of *homoleptic* derivatives, and N,O or N,N chelating ligands, in order to obtain *heteroleptic* systems (Chart 1).



M = Zn(II), Cu(II)
X,X = O,O
X,X = N,N

Chart 1 Schematic representation of bis-chelated homo and heteroleptic M(II) complexes.

In the case of the *heteroleptic* bis-chelated complexes, ionic derivatives can be obtained by using various counterions, so far adding electrostatic to the π–π stacking interactions in their possible intercalative DNA mechanism of action. Moreover, the incorporation of a transition metal ion into a recognized drug of natural extraction, introduces ionicity in the derived potential metallodrug, particularly when the overall charge is not neutralized by the coordinated ligands. According to a new point of view, the metal ion, therefore, plays the role of a carrier for the pharmacologically active biomolecules, similar to a counterion in a pharmaceutical salt or the co-former in a pharmaceutical co-crystal.[41]

In particular, we will focus on biologically active ligands where the presence of hydroxyl groups in proximity of keto group as well as β-diketone units confers metal chelating abilities, giving rise to new structural entities with modified biochemical activities. Among the bioactive metal chelating ligands of natural extraction, examples of metal-based flavonoids and tropolonoids will be given. Flavonoids are a group of polyphenolic compounds found in fruits and vegetables. They are among the most investigated phytochemicals due to their pharmacological and therapeutic activities. Their ability to chelate with metal ions has resulted in the emergence of a new category of molecules with a broader spectrum of pharmacological activities.[42] On the other hand, Hinokitiol, (2,4- isopropyltropolone, hkt) a naturally occurring α-hydroxyketone extracted from wood (Thujapalicata, Taiwan Hinoki), continually attracts interest due to its versatile biological properties (antimicrobial, insecticidal, cytotoxicity on tumour cells)[43-45] and as possible metal chelating ligand, associated to the presence of the α-hydroxycheto group.[46]

Within the field of β-diketone naturally occurring drugs, particular emphasis will be direct on the photoactive curcumin, the principal active ingredient (yellow pigment) in the traditional dietary spice turmeric (dry rhizomes of Curcuma longa, Zingiberaceae).[47] Over the past decades, curcumin has attracted interest in the fields of biology, medicine and pharmacology due to its antitumor, anti-inflammatory, antiviral, antibacterial and antioxidant activities.[48] However, one of its major drawback as effective drug is recognized to be its relatively poor bioavailability and easy oxidability and photodegradability.[49-51] In order to overcome these limitations, coordination of curcumin to metal ions, inducing consistent structural variations (i.e. the disappearance of the keto-enol group or the achievement of a more planar overall conformation) has been demonstrated to be a helpful approach.[52-54] Moreover, curcumin-based metal complexes, besides overcoming the pure curcumin drug limitations becoming suitable drug delivery carrier, can be themselves biologically active, combining in one molecule dual-action drug activities.

Selected Zn(II) and Cu(II) bis-chelated complexes

Among the great variety of potential O_2-donor bidentate chelating organic molecules, we will restrict our overview on two families of natural extraction products, flavonoids and tropolonoids (Fig. 1).

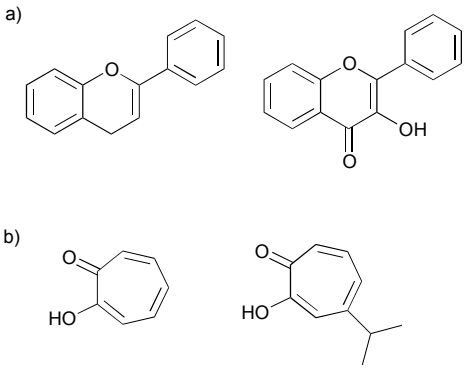


Fig. 1 Skeleton of typical flavonoids and flavonols (a) and tropolone and hinokitiol (b).

Both flavonoids and tropolonoids have been reported to possess wide-ranging pharmacological properties that include anticancer, anti-inflammatory, antiatherosclerotic, antioxidant, antidiabetic, antimutagenic, and antiviral activities.[55,56] Most probably in both cases the presence of hydrophilic phenolic hydroxyl groups and the relatively hydrophobic cyclic carbon skeleton play an important role in the interactions of these molecules with biological entities, thus influencing their physiological activities. The chelating sites in the flavonoids are the hydroxyl groups in the flavonoid framework and the keto group,[42] which make these class of organic ligands similar to trololonoids in their metal ion chelating abilities.

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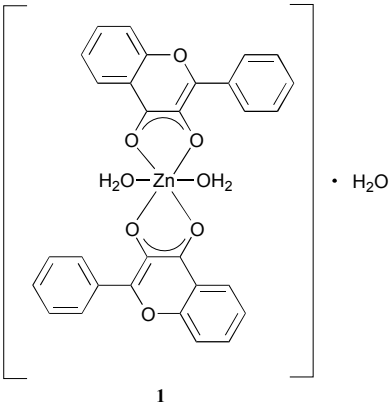
Metal-based homoleptic and heteroleptic flavonoid complexes

The limited experimental studies on flavonoids partially are due to their poor water solubility. However, flavonoids can form complexes with transition metal ions and these complexes are more hydrophilic and water-soluble than the corresponding ligands. Moreover, the antioxidant activity of flavonoids is believed to increase when they are coordinated with transition metal ions.[57] Very recently, a new homoleptic Zn(II) complex of general formula $[Zn(L)_2(H_2O)_2]$, **1**, containing the coordinated primuletin, 5-hydroxyflavone, has been synthesized and fully characterized (Fig. 2).[58]

The new complex **1** shows improved solubility properties when compared with the uncoordinated primuletin and possesses strong fluorescent properties, when excited at 429 nm, valuable for future applications in the field of DNA probes and/or diagnostic agents.



1

Fig. 2 Molecular structure of complex $Zn(L)_2(H_2O)_2]$, **1**.

Generally, flavonoids intercalate between the double-stranded DNA through hydrophobic interactions with the paired DNA bases, similarly to ethidium bromide.[59] The combination of these intercalating ability and fluorescence activity, together with the presence of the incorporated metal ions (favouring electrostatic interaction with the negatively charged phosphate backbone in the double-stranded DNA), can make the derived metal complexes an excellent tool for investigating their anticancer mechanism of action via optical methods.

Three flavonoids Cu(II) complexes containing hesperetin (Hsp), naringenin (Nrg), and apigenin (Apg) of general composition $[CuL_2(H_2O)_2]\cdot nH_2O$, **2**–**4**, were synthesized and photophysically characterized (Fig. 3).[60] The free ligands and the metal complexes have been tested in vitro against the human cancer cell lines hepatocellular carcinoma (HepG-2), gastric carcinomas (SGC-7901), and cervical carcinoma (HeLa). The results proved that the three complexes show inhibitory rates and cytotoxicity against the studied cell lines, presumably due to the different flavone overall structure (being Apg planar compared to both Hsp and Nrg) and, in the case of Hsp, related to the different hydroxyl (OH) substitutions in the rotationally free phenyl ring. The binding properties with calf thymus DNA revealed that Hsp and its Cu(II) complex **2** bind DNA mainly by intercalation mode.

In a recent study, the homoleptic Cu(II) complex $[Cu(Querc)_2(H_2O)_2]$, **5**, containing the bioflavonoids quercetin (Querc) (Figure 3) has been found to promote cleavage of plasmid DNA, producing single- and double-strand breaks, and to intercalate into the stacked DNA base pairs. The suggested antitumor mechanism of action induced by **5** consists not only in the oxidative DNA damage with reactive oxygen generation (ROS) but also in a specific interaction with DNA.[61] Indeed, its interaction with calf thymus DNA and its intercalation into the double helix of the DNA, was previously demonstrated by the use of UV–vis spectrophotometry, cyclic voltammetry and synchronous fluorescence spectroscopy with Neutral Red (NR) dye as a spectral probe.[62]

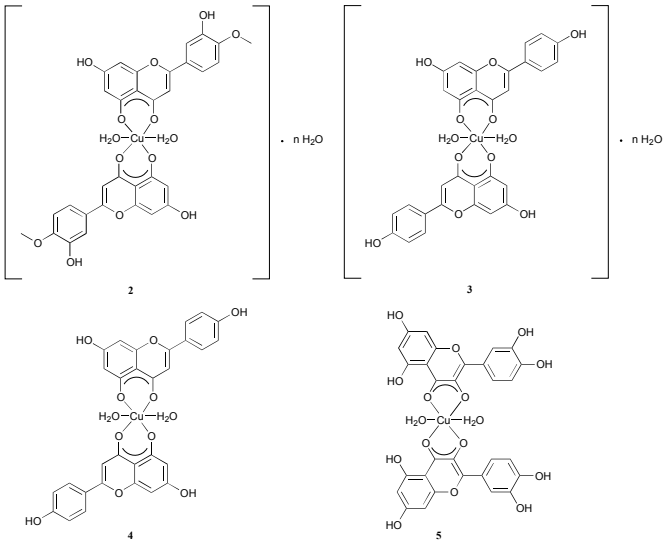


Fig. 3 Molecular structures of complexes $[CuL_2(H_2O)_2]\cdot nH_2O$, **2–5.**

Usually, the flavonoid-based metal complexes enhancement of pharmacological activities with respect to their parent flavonoids may be attributed to their ability to confer more planarity to the derived molecules, a structural feature strongly influencing their pharmacological activity.[42,63] Another important aspect that influences the biological activities of flavonoids is their interactions with the biological membrane. Non-planar structures display lesser interaction with membrane components than planar structures.[64] Therefore the improvement in the biological activities of flavonoids on complexation may be due to their ability to alter membrane fluidity and permeability. A very interesting study has just appeared in literature reporting on the mode of interaction of a very less explored flavonoid glycoside, naringin, and its metal complexes with copper, complex **6** in Fig. 4, with the cell membrane using erythrocyte as membrane mimic.[48a] The anti-cancer potential of the these molecules has been be evaluated using MCF-7 cells and normal mouse fibroblast cells.

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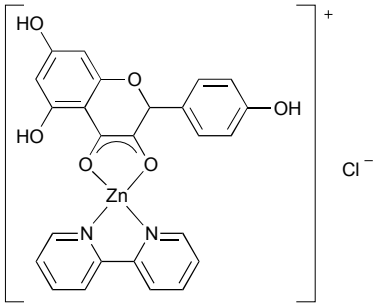

10

Fig. 4 Molecular structure of the copper naringin complex **6.**

While naringin exhibited a greater tendency to enhance membrane fluidity, its Cu(II) complex exhibited a tendency to increase the membrane rigidity. As a result, complex **6** displayed greater toxicity towards cancer cells than naringin.

Within the field of heteroleptic flavonoids metal complexes, examples of particular relevance are represented by those systems in which the ancillary ligands are N,N chelating extended aromatic molecules. Indeed, metal complexes containing phenanthroline and related ligands have been intensively investigated because of their numerous biological activities such as antitumor, antibacterial, and antimicrobial.[29,65] In this light, DNA binding and cleaving abilities of four copper and zinc complexes of general formula [Cu(Kae)(phen)]Cl·2H_2O, **7**, [Cu(Kae)(bpy)]Cl·H_2O, **8**, [Zn(Kae)(phen)]Cl·H_2O, **9**, and [Zn(Kae)(bpy)]Cl·H_2O, **10**, where Kae = kaempferol, phen = 1,10-phenanthroline, bpy = 2,2'-bipyridine were studied and their antitumor activity investigated in vitro (Fig. 5).[66]

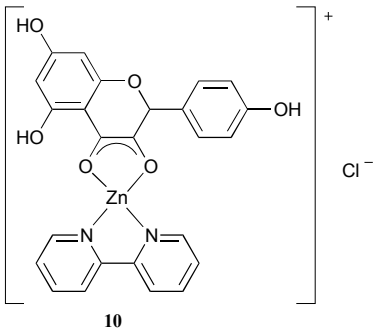

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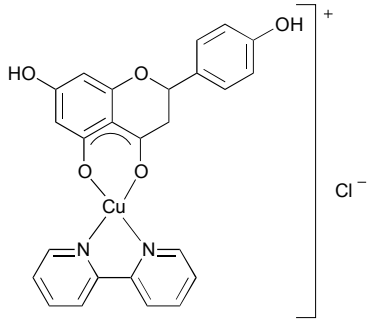
Fig. 5 Molecular structure of complex [Zn(Kae)(bpy)]Cl H_2O, **10**.

The binding and cleavage abilities of complexes **7**–**10** with DNA have been studied by fluorescence spectroscopy, viscosity measurements, and gel electrophoresis under physiological conditions. The experimental results indicated that the four new complexes could bind to Calf Thymus DNA (CT-DNA) via an intercalative mode, complex **7** showing the strongest ability to inhibit the growth of human breast carcinoma cells (MDA-MB-231). Moreover, a recent study on the effects of Cu(II) and 2,2′-bipyridine complexation with naringenin on MDA-MB-231 breast tumor cells, has demonstrated that the new complex [Cu(Nrg)(bpy)]Cl, **11**, is more efficient inhibiting colony formation, proliferation and migration than naringenin itself (Fig. 6).[67]

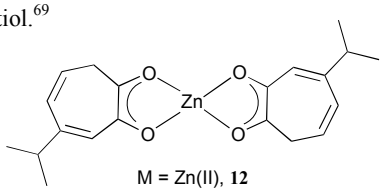


11

Fig. 6 Molecular structure of the complex **11.**

Metal-based homoleptic and heteroleptic tropolonoid complexes

Many metal derivatives of both hinokitiol and its parent tropolone have been prepared in literature and the possible synergism between the tropolonoids biological properties and the presence of the metal ion analyzed.[68] Indeed, the Zn(II) homoleptic complex [Zn(hkt)$_2$], **12**, shown in Fig. 7, has been found to show in vitro anti-diabetic and insulin-mimetic properties, enhanced when compared to the pure organic hinokitiol.[69]

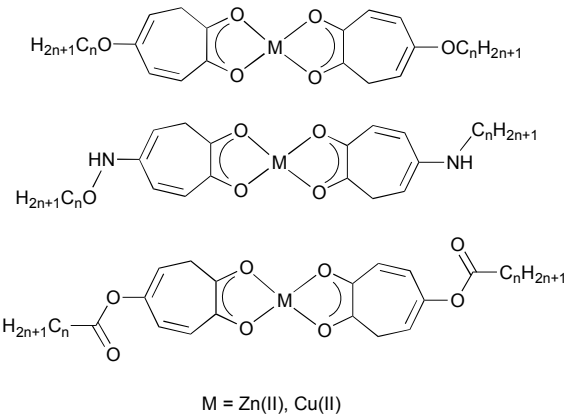


M = Zn(II), **12**
M = Cu(II), **13**

Fig. 7 Molecular structures of complexes **12** and **13.**

Moreover, it has been reported that both complexes **12** and the Cu(II) analogue [Cu(hkt)$_2$], **13**, induce apoptotic cell death in various tumor cell lines and are potent antitumor agents for cancer cells including malignant melanomas.[70]

Between the various properties shown by both tropolonoids and their metal derivatives, of particular interest is their ability to organize themselves into liquid crystalline phases. The introduction of suitable substituents on the tropolonoid aromatic rings and the subsequent metal coordination, has lead to a class of Zn(II) and Cu(II) bis-tropolonates complexes with liquid crystalline properties (Fig. 8).



M = Zn(II), Cu(II)

Fig. 8 Molecular structures of functionalized metal-based tropolonoid complexes showing liquid crystalline properties.

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Indeed, a new class of metallomesogens (metal containing liquid crystals) have been obtained, where the tropolonate ligands contain, in the 5 position of the aromatic ring, long aliphatic chains able to induce the liquid crystalline aggregation state.[71]

The coordination environment around the central metal ion in heteroleptic metal-based tropolonate complexes plays an important role in the derived biological activities of the resulting complexes. Good examples are some Zn(II) heteroleptic complexes containing as secondary chelated ligand the 2,2'-bipyridine-4,4'-dinonyl-2,2'-bipyridine (bpy-9) (Fig. 9).[29]

The molecular structure of **16** has been obtained by single crystal X-ray analysis (Fig. 10a), showing the distorted octahedral geometry coordination around the zinc(II) ion, while the neutral nature and, therefore, the five-coordination of the zinc ion in complexes **14** and **15**, has been confirmed subsequently by the single crystal X-ray molecular structure of an analogous tropolonate complex containing as N,N chelated ligand the 4,4'-bis-(hydroxymethyl)-2,2'-bipyridine (Fig. 10b).[72]

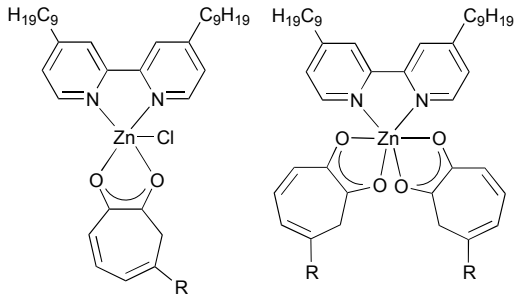

Fig. 9 Molecular structures of the tropolonoid zinc derivatives **14**-**17**.

The uncoordinated ligands as well as the corresponding Zn(II) complexes **14-17** have been tested *in vitro* towards three different human prostatic cancer cell lines: DU145, LNCaP and PC-3. While almost all ligands are inactive, most of the Zn(II) complexes show promising anticancer properties. Moreover, the activitiy of all Zn(II) complexes towards PC-3 cells is not strongly related to the stoichiometric ratio of the tropolonate ligands, being almost the same for the mono and the bis-diketonate species. As an important result from this study, the nature of the central metal ion induces significant changes in the biological activities of the resulting complexes.

Indeed, comparing complexes **14-17** with Pd(II) and Pt(II) heteroleptic mono tropolonate derivatives or with homoleptic Zn(II) bis-diketonates,[46b,53] it has been possible to conclude that the simultaneous presence of flat chelating ligands around the Zn(II) ion as metal scaffold has been a winning choice for endowing with good antitumor activity in new non platinum complexes.

Zn(II) and Cu(II) curcumin and curcuminoids complexes

Curcumin, 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curc), is a hydrophobic polyphenol, existing in different tautomeric form (Fig. 11). Although curcumin possesses wide-spectrum and low-toxicity antitumor activity, its clinical application in antitumor therapy has been greatly limited by its rapid metabolism, low absorption and poor bioavailability and stability in vivo.[48,73] In order to improve the bioavailability and antitumor activity of curcumin, a large number of curcumin derivatives and analogues have been designed and synthesized through structural modifications such as variation of the aromatic rings and their substituents and/or replacing the heptadiendione bridge chain of curcumin with other linkers.[74]

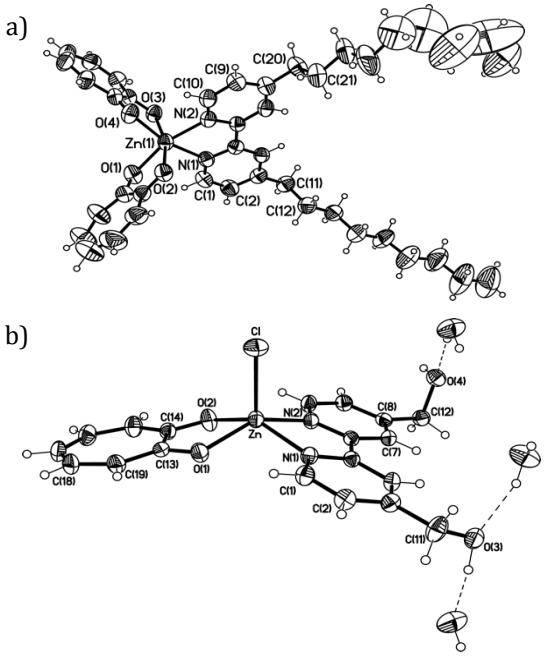

Fig. 10 X-ray molecular structures of complex **16** (a) and complex **14** analogous containing the 4,4'-bis-(hydroxymethyl)-2,2'-bipyridine (b).[72]

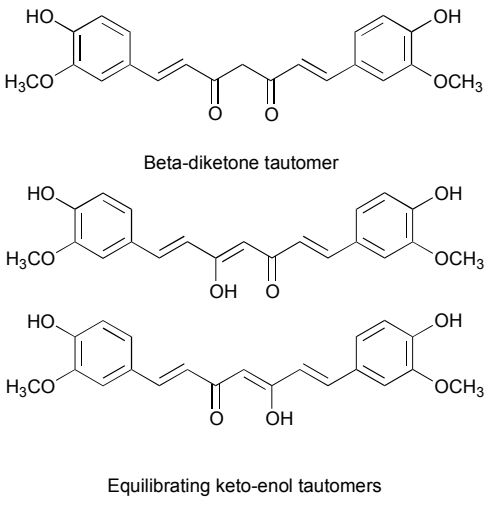

Fig. 11 Tautomeric forms of curcumin.

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One of the recent structural modification concerns the introduction of a metal ion within the β-diketone unit, with the double intent to prevent degradation through stabilization and to enhance the biological activity through induction of planarity within the curcumin moiety. Indeed, it has been demonstrated that the coordination of curcumin with various metals can enhance the antitumor activity.[53a,b,75] Within this field, curcumin complexes containing the low cost Zn(II) and Cu(II) metal ions are, now days, of particular interest and several paper appeared reporting their promising cytotoxic activity against different human cancer cells as well as their potential use as diagnostic agents due to their intrinsic fluorescence properties.

The most studied metal-based curcuminoid derivatives are of the homoleptic type.[76] Although β-diketones are known to form complexes with almost every metal, not many zinc and copper complexes of curcumin and derivatives have been reported and evaluated as anticancer agents and most of them, initially, were based on bis-M(curc)$_2$ with the general chemical structure reported in Chart 2.[77]

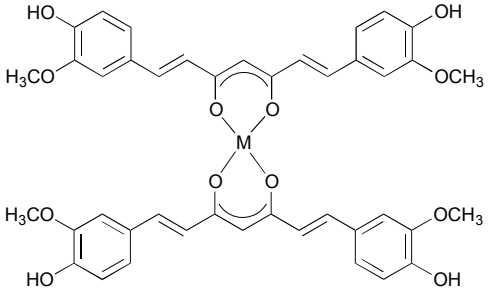

Chart 2 General chemical structure of homoleptic bis-M(curc)$_2$ based complexes.

In the case of copper, it has been observed that complexation with curcumin reduces its toxicity and gives rise to some new metal-based antioxidants.[78]

Very recently homoleptic copper complexes of some curcumin derivatives have been reported in literature. In particular, since the poor stability of curcumin is mainly attributed to the phenolic hydroxyl groups,[79] a series of dialkoxyl substituted curcumin derivatives has been synthesized. The new curcumin derivatives have been used as ligands in the preparation of two new bis-Cu(curc-deriv)$_2$, complexes **18** and **19**, whose X-ray molecular structures are reported in Fig. 12.[80,81]

Cytotoxicity of the free ligands and their Cu(II) complexes **18** and **19** against three cell lines, ASPC-1, MCF-7 and HeLa were evaluated. For both complexes, their cytotoxic activity was found about three–fivefold higher than the corresponding free ligands against every cancer cell tested. Moreover, complex **18** exhibited higher antiproliferative activity against the three human cancer cell lines tested than complex **19**, explained by the author as the increased steric hindrance in the substituents groups brings down the antitumor activity.

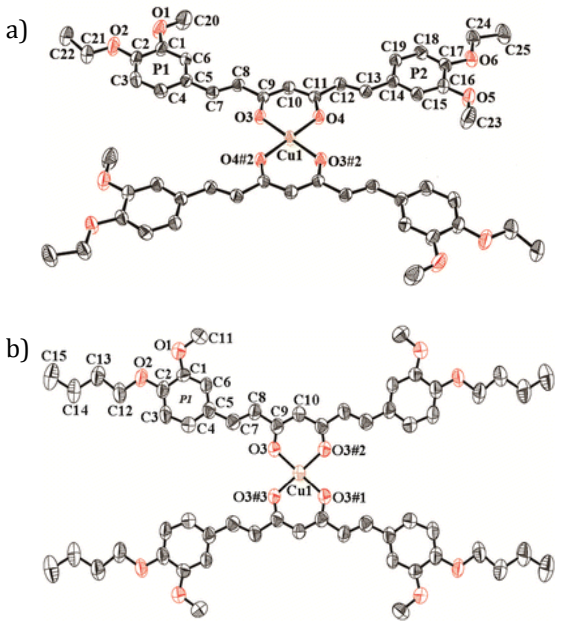

Fig. 12 Single crystal X-ray molecular structures of complexes **18**(a) and **19** (b) with atomic numbering scheme.[80,81]

Complex **18** and its crystal structure has also appeared in another interesting paper, where the possible use of this new curcumin metal based derivative as probe for in vivo imaging, is demonstrated.[80] Complex **18** has shown more intense one and two-photon excited fluorescence (1PEF and 2PEF), a larger two-photon absorption cross-section in the NIR region, higher quantum yield and photostability and lower cytotoxicity against the human breast cancer MCF-7 cell line when compared to the pure curcumin ligand derivative. The tumor targeting capability of **18** on tumor-bearing nude mice *in vivo* has proved that this complex is able to target the tumor for fluorenscence tumor imaging, the fluorescence intensity remaining significant for 8/10 hours (Fig. 13).

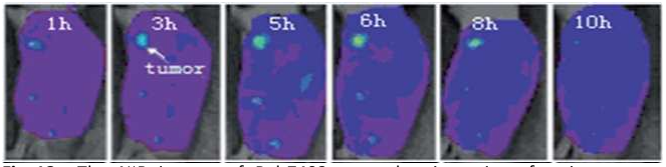

Fig. 13 The NIR images of Bel-7402 tumor-bearing mice after intravenous injection of complex **18**.[81]

One of the first Zn-curcuminoids molecular structure appeared in literature few years ago, where zinc binds two 9Accm ligands (9Accm=1,7-(di-9-anthracene)-1,6-heptadiene-3,5-dione) and one pyridine molecule, [Zn(9Accm)$_2$(py)], **20**, (Fig. 14).[82]

Complex **20** can be considered an homoleptic Zn(II)-based curcuminoid derivative, with the difference that, at least in the crystalline solid state, one molecule of pyridine complete the metal ion coordination sphere.

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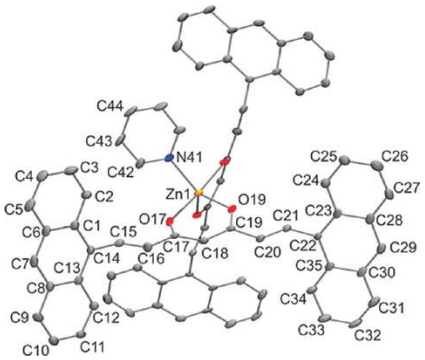


Fig. 14 Single crystal X-ray Molecular structure diagram of complex **20** with atomic numbering scheme.[82]

Interestingly, complex **20** displays a quasi-ideal bipyramidal trigonal arrangement unlike the similar copper compound, [Cu(9Accm)$_2$(py)], **21**, which shows a square pyramidal environment, as proved by its single crystal X-ray structural analysis (Fig. 15).[54]

The photophysical properties of both compounds **20** and **21** have been deeply investigated. As shown in Fig. 16, fluorescence assays in the same solvent indicate that **20** exhibits enhancement of fluorescence while complex **21** an enhancement of quenching with respect to the fluorescence response of the free ligand, proving that 9Accm has a high-sensitivity response upon coordination. However, when compound **21** was tested against a variety of human tumor cell lines, it has exhibited hardly any activity.

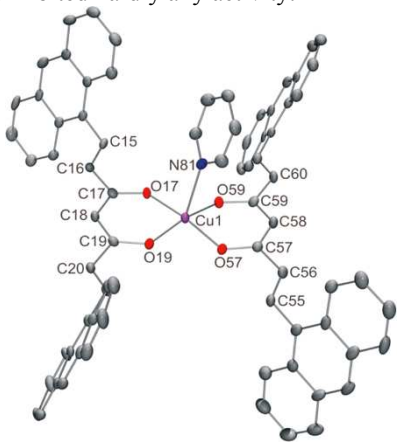


Fig. 15 The X-ray molecular structure of complex **21** with atomic numbering scheme.[54]

Another interesting class of atypical homoleptic zinc and copper curcumin complexes is represented by some binuclear curc-M(II)-μ-hydroxo complexes, obtained by the reaction of the metal(II) nitrate and curcumin in a 1:2 molar ratio and characterized for their antitumor activity towards different cancer cell lines (Fig. 17).[83]

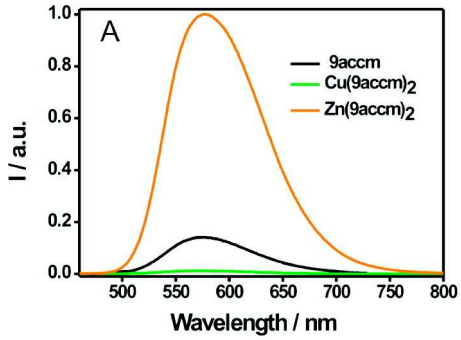
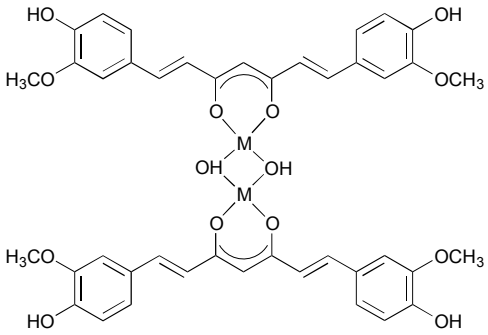


Fig. 16 Normalized emission spectra of 9Accm, complexes **20** and **21**.[82]

In contrast with the accepted idea that there exist a synergistic enhancement of ligand effects by complexation with metal ions, in the case of complexes **22** and **23**, tested against a panel of human tumor cell lines (MCF-7, HePG2, Hela, HCT-116) the percentage of cell survival has shown that the curcumin alone is more active than the M(II)-curcu based complexes.

Since curcumin has been shown to bind in the minor groove of nucleic acids, due to its overall molecular shape, typical for minor groove binders,[84] its metal based derivatives can, potentially, interact with DNA non-covalently by intercalation or groove binding. Moreover, molecules with extended planar aromatic systems can be inserted between paired DNA bases through π–π stacking, affording significant unwinding, stiffening and lengthening of the helix and therefore behaving as intercalators. Most of the known metallo-intercalators are



M = Zn(II), **22**
M = Cu(II), **23**

Fig. 17 Molecular structures of curc-M(II)-μ-hydroxo complexes **22** and **23**.

typically either coordinatively saturated square planar Pt(II) or octahedral Rh(III) and Ru(II) complexes based on aromatic nitrogen ligands. These compounds are extensively used as structural probes for DNA or as diagnostic agents rather than as anticancer agents.[85] However, through the combination of the chelating capabilities of both curcumin or curcuminoids and some aromatic nitrogen ligands such as bipyridines or phenanthrolines, new heteroleptic curcumin-based Zn(II) and Cu(II) have been designed. Doing so, the coordination sphere of the metal ion is filled by an intercalating ligand and a chromophore unit, with the generation of metal complexes in which there is a combination of intercalating ability, potential cytotoxic activity and fluorescence.

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Recently, the synthesis, the characterization and the biological evaluation of two heteroleptic complexes (**24** and **25** in Fig. 18a) containing curcumin and a 2,2'-bipyiridine ligand, substituted in 4,4'- positions with different groups (dinonyl-2,2'-bipyridine, bpy-9 or 4,4'-bis-(hydroxymethyl)-2,2'-bipiridine, bpy-OH), tethered around the Zn(II) ion, have been reported.[33]

The neutral nature of both **24** and **25** complexes has been confirmed both in solution (conductivity measurements) and in the solid state. Indeed, the molecular structure of **24**, obtained through X-ray diffraction analysis, has proved that the Zn(II) ion is in a five-coordinated distorted square pyramidal geometry, where the basal plane is formed by the two chelating ligands, bipy-9 and curc, respectively, while the chloride ion is located on the apex of the pyramid (Fig. 18b).

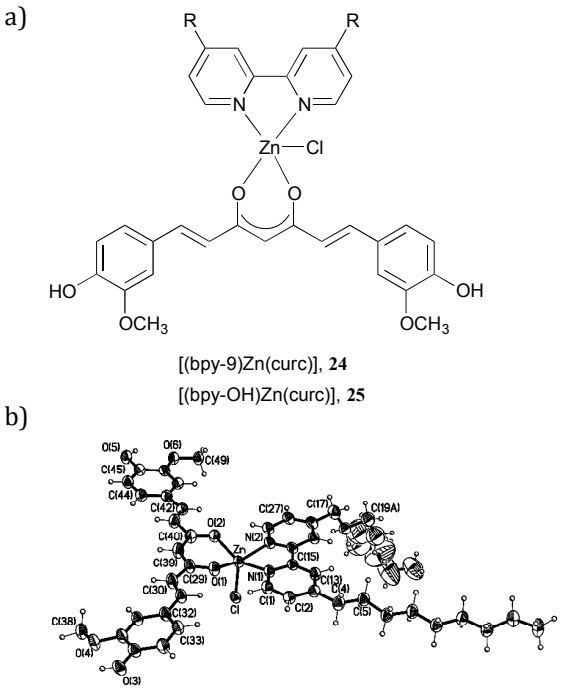

Fig. 18 Proposed structure for complexes **24** and **25** (a) and single crystal molecular structure with atomic numbering scheme of complex **24** (b).[33]

Fluorescence has been detected for both complexes as well as significant cytotoxic activity towards a panel of human cell prostate cancer cells (DU145, LNCaP and PC-3). In particular, the curcumin Zn(II) complex **24** shows the strongest growth inhibition in all cell lines, being even more effective than the pure curcumin in the LAN-5 neuroblastoma cell line. The intrinsic fluorescence of **24** and **25**, made possible investigation of their interaction with DNA through a new optical method (based on the anisotropy of the optical molecular properties such as absorbance and fluorescence of DNA oligomers in orientationally ordered liquid crystal domains) with the reference fluorescent intercalator ethidium bromide. This analysis demonstrates that the interaction mode of curcumin, complexes **24** and **25** with the DNA the double helix favours their alignment perpendicular to the DNA axis, suggesting a partial inter-base intercalation of these Zn(II) complexes. The intrinsic fluorescence of complex **24** has made possible to evaluate its intratumoral distribution in an orthotopic model of glioblastoma in mice through in vivo studies.[86] Complex **24** has been found to induce conformational changes in p53-R175H and -R273H mutant proteins, two of the most common p53 mutations, therefore reactivating specific mtp53 proteins in an orthotopic U373 glioblastoma model. Moreover, the ability to reach glioblastoma tissues of the ortothopic mice model highlighted its ability to cross the blood-tumour barrier.

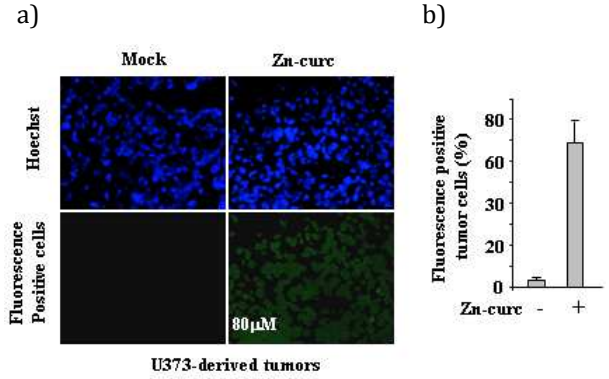

Fig. 19 Mock- or complex **24**-treated U373M-derived tumors harvested and analyzed with a fluorescent microscope (a) and quantification of tumor cell fluorescence positivity (b).[86]

To evaluate the intra-tumour localization of complex **24**, glioblastoma untreated or treated tissues were harvested and analyzed with a fluorescent microscope revealing a diffuse fluorescence into the glioblastoma tissues treated with **24**, compared to the Mock-treated tumors (Fig. 19a), as also evidenced by quantification of the fluorescence positive cells (Fig. 19b). A deep photophysical investigation of complex **24** has allowed to evaluate its *in vitro* binding to Human Serum Albumin (HAS), a protein carrier of many drugs in the blood stream.[87] Due to Serum Albumin intrinsic fluorescence, drug-HAS interaction is properly studied by checking its emission quenching subsequent to the binding of the drug within the protein hydrophobic site.[88] Indeed, complex **23** added to HAS emission wavelength shift confirmed the localization of the complex within the hydrophobic pocket of the protein, while the luminescence quenching of the Serum Albumin proved a fluorescence-resonance energy-transfer from protein to complex. The calculated value of the bimolecular quenching rate constant indicated that the quenching was due to an adduct formation, rather than to a dynamic collision.

By changing the N,N chelating unit, new Zn(II)-curc based heteroleptic complexes (**26-28**) have been reported, in which the Zn(II) ion shows the same pentacoordination seen in complex **24** (Fig. 20).[89] Therefore, the cytotoxic effects of the newly synthesized Zn(II) curcumin-based complexes **26-28** have been investigated on human neuroblastoma cell line SH-SY5Y, in a bio-hybrid membrane system, able to support cell adhesion, proliferation and differentiation.[90]

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The cytotoxic activity of complexes **26-28** has been compared with that of **24** against the same human neuroblastoma cell line SH-SY5 (Table 1).

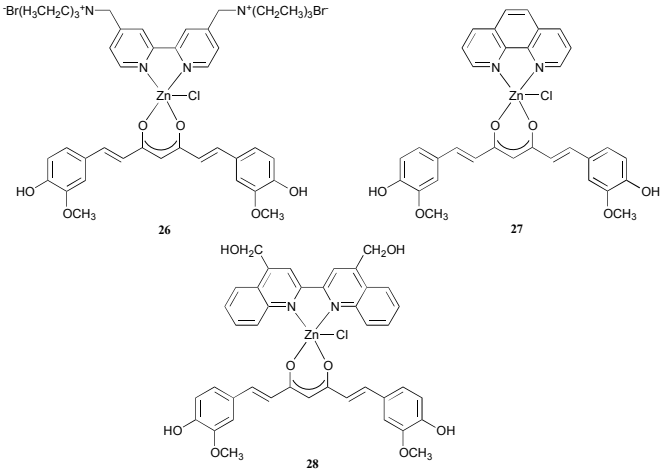

Fig. 20 Molecular structures of the new Zn(II) curcumin-based complexes **26-28.**

Table 1 IC50 values of Zn(II)-curcumin based complexes calculated on average values of % inhibition at various molecule concentrations

Complexes	IC50 μM - 24 h	IC50 μM - 48 h
23	24.36	11.79
25	30.08	19.31
26	8.75	6.84
27	18.85	20.33

The activity of complex **27** at low concentrations is remarkable also after 48 hrs of treatment. Moreover, complex **27** has a dose-dependent antiproliferative effect on cells, as demonstrated by the decrease of viability and the increase of Annexin V and PI- positive cells with the increase of its concentration (Fig. 21). As cells progress through apoptosis, the integrity of the plasma membrane is lost, allowing PI to penetrate and label the cells with a strong yellow-red fluorescence. The percentage of Annexin V and PI- positive cells markedly increased when cells were treated with high concentration of complex **27**, with a maximum of fluorescence intensity for Annexin V and propidium iodide detected at concentration of 25 μM. Both complexes **29** and **30** have been used for *in vitro* anticancer activity evaluation studies against human cancer cell lines hepatocellular carcinoma, HePG2 showing better inhibitory activity than curcumin (IC_{50} values of 7.5 and 5.6 μg, respectively, with respect to the curcumin value of 19.1 μg).

Interesting Cu(II) complexes analogous to derivative **27** have been recently reported, in which structural variation on both 1,10-phenanthroline and curcumin ligands have been introduced.

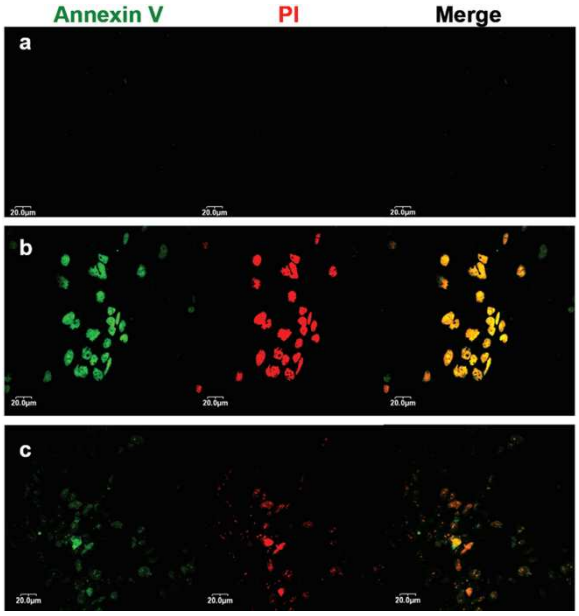

Fig. 21 LCSM images of SHSY5Y cells stained with Annexin V-FITC and PI after 24 h of culturing under normal conditions (a); under treatment with Zn(II) complex **27** at 8 μM (b) and 25 μM (c).[89]

While conductivity measurements indicate the neutral nature of all Zn(II) compounds **26-28** also in solution, leading to assume a penta-coordination of the Zn(II) ion, with two chelating units, the N,N and the O,O-curcumin ligands, and an apical chloride ion, ionic analogous of complex **26** of both zinc and copper have been synthesized, having nitrate as counterion (Fig. 22).[91]

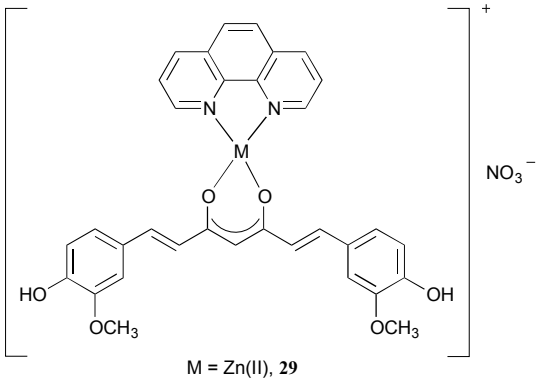

Fig. 22 Molecular structures of the ionic derivatives **29** and **30**.

In particular, when bathophenanthroline has been used as N,N chelating ligand, complex **31** has been isolated and binding mode studies with CT-DNA performed (Fig. 23).[92]

Electronic absorption spectroscopy is one of the most useful techniques for studying the binding mode of metal complexes to DNA.[93]

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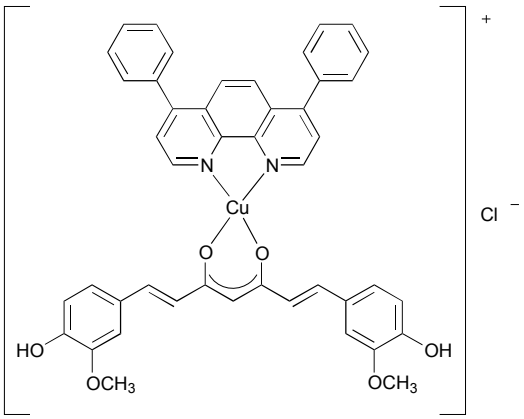

31

Fig. 23 Molecular structure of the bathophenanthroline complex **31**.

In particular, as shown in Fig. 24, the absorption spectra of complex **31** in the absence and presence of DNA, showed a maximum at 297 nm and a minimum band a 265.

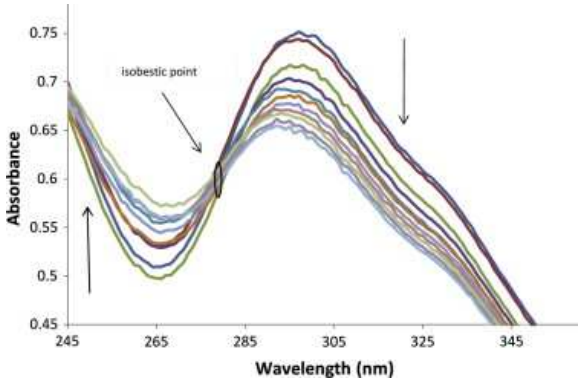

Fig. 24 Absorption spectra of complex **31** (10^{-5} M) in the absence and presence of increasing amounts of CT-DNA.[92]

As stated by the authors, all the observed changes in the absorption spectra on increasing CT-DNA concentration are consistent with the intercalation of complex **31** into the double helix of DNA with the formation of a new complex-DNA adduct.[94]

When 1,10-phenanthroline has been used as N,N chelating ligand, while, the Cu(II) ion has been chelated by the new curcuminoid ligand 9Accm, complex **32** has been synthesized and structurally characterized (Fig. 25).[53]

Differently than the analogous homoleptic complex **21** (Fig. 15), complex **32**, when tested against the same panel of human tumor cell lines, showed high cytotoxic activity, in the same range or even better than cisplatin. The difference between the two compounds has been attributed to the ability of complex **32** to exchange chloride ion in aqueous solution, becoming charged, more soluble and, therefore, more reactive.

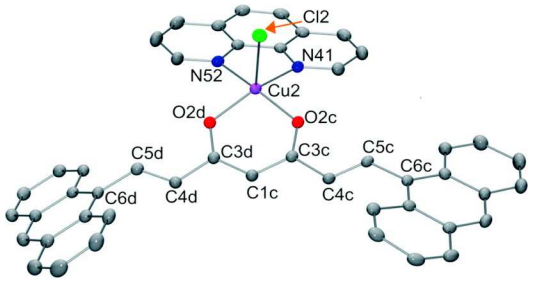

Fig. 25 The single crystal X-ray molecular structure of complex **32** with atomic numbering scheme.[54]

Taking advantages of the fluorescence properties observed in compound **32**, *in vitro* studies by using U2OS (human osteosarcoma) cells were designed to analyze its mechanism of action. Incubation of complex **32** with U2OS cells showed accumulation and fluorescence in vacuoles, outside the nuclei (Fig. 26).

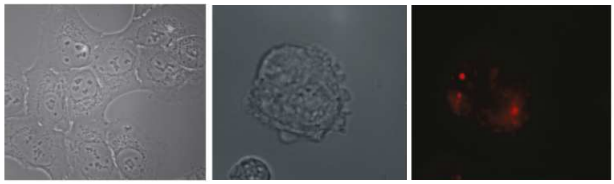

Fig. 26 U2OS cells in aqueous media before treatment (a), after 1h of incubation with **32** at 37°C (b) and fluorescent image of the treated cell (c).[54]

This outcome has suggested that, probably, DNA may not be the primary target of complex **32** and its activity involves additional or completely different type of reactions once introduced into cells. Surprisingly, the biophysical studies performed on complex **32** (Circular Dichroism, UV-vis titration with CT-DNA) agreed with a lack of intercalation between the anthracene groups and the DNA, rather pointing towards a mechanism of action based on week and electrostatic interactions and, therefore, leading to a new class of metal anticancer drugs.

Conclusions

Recently the design of various kinds of metal-based drugs containing bioactive molecules of natural extraction have attracted intense research interest as both new drug delivery systems and theragnostic agents.[39,95] Generally, within the field of cancer treatment, the metallo-drug engineering is interestingly evolving towards compounds preferably targeting DNA through non covalent modes of action such as groove binding, phosphate clamps, insertion or intercalation. In particular, the intercalation mode induces the most dramatic changes in the DNA structure. Molecules with extended planar aromatic systems are suitable candidates to insert between paired DNA bases through π–π stacking as well as other type of intermolecular interactions, such as hydrogen bonding, due to the presence of suitable periphery substituents. In this contest,

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we have summarized some of the recent developments in the construction of Zn(II) and Cu(II) complexes, containing a sufficient number of aromatic planar fragments and/or suitable hydrogen bonding sites, to be potential intercalating metal agents, specifically built up through the choice of O,O chelating ligands bioactive and of natural extraction. The chelating ability of molecules belonging to families such as flavonoids, tropolonoids and curcuminoids are becoming well known over the years as well as the advantages deriving from their coordination to metal ions. Often, especially with Zn(II) and Cu(II), the derived complexes are more hydrophilic and water-soluble than the corresponding ligands, overcoming some of the major drawback in the use of these natural products as effective drugs. Their potential intercalative mechanism of action is maximized upon chelation to metal ions, due to the planarization of the entire molecule, the more extended π electron delocalization obtained with the formation of the metallacycle,[96] and the addition of electrostatic interactions intrinsic to the presence of the metal ion.[10] Moreover, fluorescence properties often induced by the presence of the metal ion in these new adducts, gives the opportunity to deeply investigate their mechanisms of action through optical methods, improving the knowledge of structure activity properties and adding the opportunity to be used as diagnostic agents.

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Notes and references

† This article is dedicated to the memory of Daniela Pucci, friend and colleague of inestimable value, who strongly believed that our small contribution against a huge battle such as cancer would have been of help for so many people suffering, but never losing their hope. Daniela hoped until the end.

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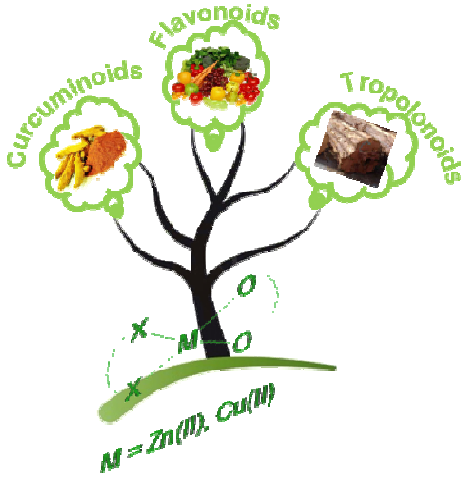
Zn(II) or Cu(II) highly stable complexes with chelated O,O-donor ligands of natural extraction giving rise to delivery drug systems, new biologically active complexes and potential diagnostic agents due to their intrinsic spectroscopic properties.



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