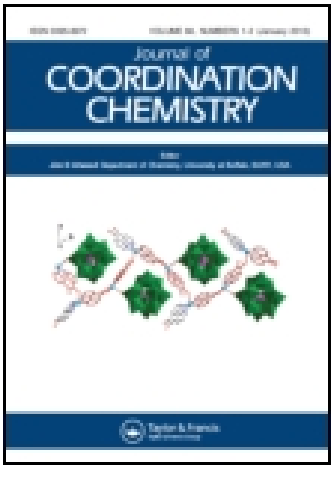

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Copper compounds in nuclear medicine and oncology

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REVIEW

Copper compounds in nuclear medicine and oncology

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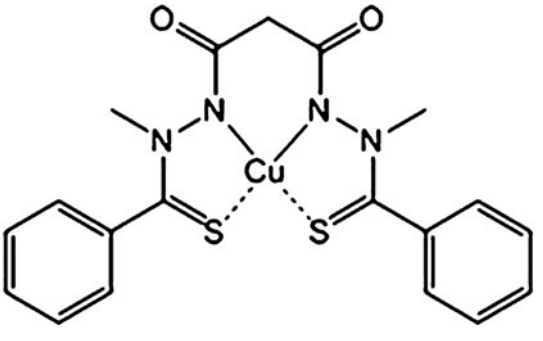

There have been some notable developments in several areas of inorganic pharmaceuticals that have potentially far-reaching importance for future medical applications and research. One significant development in the field of oncology and hematology is the application of copper complexes. Copper ions play an important role in biological systems, and without their catalytic presence in trace or ultra trace amounts many essential co-factors for biochemical reactions would not take place. Copper is an essential component of several endogenous antioxidant enzymes. Copper affects the transcription of multiple defense and repair genes to protect against metal-induced pathologies. Copper creates stable complexes with a wide variety of organic molecules that can provide required biological affinity and therapeutical activity suitable for targeting specific locations in the body (when using radioactive copper isotopes, ^{60}Cu, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu), as well as non-radioactive copper. The aim of this review is a critical evaluation of copper complexes used for therapy or diagnosis of various diseases in oncology from 2000 to the present.

Keywords: Copper complexes; Oncology; Copper homeostasis; Coordination chemistry; Radiopharmaceuticals

1. Introduction

Medicinal inorganic chemistry is one of the most rapidly developing areas of pharmaceutical research [1]. One of the fundamental goals in medicinal chemistry is the development of

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new anticancer and antimicrobial therapeutic agents. Cancer treatment using metal-based drugs is one of the very effective strategies as the metal ions are capable of binding to nucleic acids stereo-specifically with varying strength [2].

Copper being an essential metal for animals and non-toxic in controlled quantity is implicated in many diseases [3]. The toxicity of metal atoms is due to reactions of redox-active metal ions, which generate reactive oxygen species (ROS) that damage DNA and other biomolecules, probably *via* Haber–Weiss or Fenton-like reactions [4]. The accumulation of copper is observed to be a physiological feature of many tumor tissues and cells and it was demonstrated in many types of human cancers including breast, prostate, colon, lung, and brain [5]. However, there are several metal–organic compounds that actively and specifically inhibit the chymotrypsin-like activity of the proteasome *in vitro* and in human tumor cell cultures. Copper(II) complexes have been extensively utilized in DNA cleavage for generation of activated oxygen species by redox cycling properties between Cu(II) and Cu(I), presumably a hydroxyl radical, which can react at several nucleic acid sites to break it [6]. Copper(II) complexes with N and O donors have also been studied for their nuclease properties [7]. Copper accumulates in tumors due to the selective permeability of the cancer cell membrane to copper compounds. For these reasons, a number of copper complexes have been screened for their anticancer activity and some of them were found active both *in vivo* and *in vitro* [8, 9].

2. Body copper homeostasis

Knowledge of copper distribution, transmission, absorption, and elimination mechanisms in the body is important for the proposal of diagnosis and treatment strategies, when using copper and its complexes. The chemical properties which make copper biologically useful can also potentially lead to toxic effects, namely, copper-induced oxidative stress. The complex mechanisms of copper transport and metabolism in order to maintain copper homeostasis are underscored by two inherited disorders, Wilson's disease and Menke's disease [10].

Copper is an essential trace element with many physiological functions. It affects activity of many enzymes (Cu/Zn-superoxide dismutase (Cu/Zn-SOD), ceruloplasmin, cytochrome oxidase, tyrosinase, dopamine hydroxylase, and lysine oxidase), both as a co-factor and as an allosteric component. These enzymes are essential for cellular respiration, defense against free radicals, melanin synthesis, formation of connective tissue, and for iron metabolism. Copper-dependent transcription factors play an important role in gene expression. A daily dietary intake of 1–2 mg copper seems to be necessary to enable copper-dependent metabolic processes in adults. The main sources of dietary copper are dried beans, nuts, shellfish, and liver. Copper is absorbed readily from the stomach and small intestine. The liver is the major location of stored copper, containing about 10% of the total body content. In blood serum, copper is transported mainly by ceruloplasmin and, in smaller part, by histidine and albumin [11].

Genetic studies in model systems and biochemical, cell biology, and structural studies have identified many proteins involved in eukaryotic copper homeostasis and provided insights into their structures and mechanisms of action. It is likely that there are other functions and mechanisms to be discovered for known copper homeostasis proteins, and new components of this machinery will be discovered and integrated into our current understanding of cellular copper balance [12]. Many questions have yet to be tackled in the field

of copper metabolism, from new, exciting, and perhaps serendipitous perspectives. How does copper make its way from the site of import at the plasma membrane, or efflux from intracellular storage vesicles, to the copper chaperones and other intracellular molecules? What is the entire constellation of copper-binding proteins in a mammalian cell? How do the components of the copper homeostasis machinery communicate, and what other signals, including chemical, hormonal, and environmental, trigger changes in copper homeostasis? How do multicellular organisms sense copper deficiency in peripheral tissues and transmit this signal to mobilize copper stores [13].

Body Cu levels are the result of a balance between Cu absorption and Cu excretion by the bile. Although important advances have been made in understanding of Cu excretion, the mechanisms that govern intestinal Cu absorption remain largely a mystery. In humans and other mammals, Cu is absorbed primarily by the small intestine. Although about the same net amounts of dietary Cu and Fe are absorbed daily by humans, the actual amount of Cu absorbed is about four times higher, since a great deal of Cu recycles between the intestinal tract and the organs that supply it with secretions [14]. The mechanism by which Cu enters the cells of the intestinal mucosa and crosses into the interstitial fluid and blood is not well understood. Earlier studies indicated that uptake of Cu^{2+} across the brush border involved both a non-energy-dependent saturable carrier active at lower Cu concentrations, and diffusion at higher concentrations. Although Ctr1 (Cu transporter 1) is the default intestinal Cu transporter, it was demonstrated that Cu^{+} is also transported by divalent metal transporter 1 (DMT1) [15]. Intestinal (Caco-2 cells), hepatic (HepG2 cells), and kidney (Hek293 cells) cells regulate the uptake of Cu. In intestinal Caco-2 cells, it was found that pretreatment with excess Cu enhanced uptake and overall transport of 1 μM ^{64}Cu, a response opposite to what would be expected for homeostasis [16]. In contrast, Cu-depleted cells responded by markedly increasing their uptake and overall transport of copper. These results suggest the possibility of a biphasic response to Cu concentration, with transport increasing at both low and high intracellular concentrations of copper [17].

Within the mucosal cell, most newly absorbed Cu (about 80%) is retained in the cytosol, the majority of which is bound to metallothioneins (MTs) and/or proteins of similar size. Since MTs have high affinity for copper, and their expression is largely increased under high Cu conditions, it is possible that under elevated Cu conditions inflow of Cu will be driven by cytosolic MT binding.

Cellular Cu homeostasis seems to be tightly controlled by the activity of MTs and chaperones, with practically no free ionic Cu in the cytosol. In contrast to iron, Cu has an effective mechanism of excretion, which allows for adequate control of its body homeostasis [18].

In addition to delivery of copper to cytosolic proteins and to the secretor compartment, copper must be targeted to mitochondria, where cytochrome oxidase uses copper for oxidative phosphorylation. Genetic studies in microbes and the mapping of mutated genes that are responsible for defects in cytochrome oxidase assembly have identified many proteins involved in cytochrome oxidase copper metallation [19].

3. Copper and its radionuclides

Copper-based radionuclides (table 1) are currently being evaluated as ideal radioisotopes for positron emission tomography (PET) imaging (^{64}Cu, ^{62}Cu, ^{60}Cu, and ^{61}Cu) and

Table 1. Characteristics of copper radionuclides [21].

Isotope	$T_{1/2}$	β^- MeV (%)	β^+ MeV (%)	EC (%)	γ MeV (%)
^{60}Cu	23.4 min	–	3.92(6) 3.00(18) 2.00(69)	7.4	0.85(15) 1.33(80) 1.76(52) 2.13(6)
^{61}Cu	3.32 h	–	1.22(60)	40	0.284(12) 0.38(3) 0.511(120)
^{62}Cu	9.76 min	–	2.91(97)	2	0.511(194)
^{64}Cu	12.8 h	0.573(39.6)	0.655(19.3)	41	1.35(0.6) 0.511(38.6)
^{67}Cu	62.0 h	0.577(20) 0.484(35) 0.395(45)	–	–	0.184(40) 0.092(23)

radiotherapy (^{67}Cu and ^{64}Cu), and the well-established coordination chemistry of copper allows for utilization of a variety of chelate systems that can be linked to biologically relevant molecules. PET imaging is now routinely used in the clinic, but it will not reach its full potential in oncology before significant advances are made with longer lived PET isotopes, such as ^{64}Cu. ^{64}Cu is one of the few useful metallic positron-emitting radionuclides with a relatively long half-life (12.7 h), permitting studies to be performed for as long as 48 h after administration. Moreover, because ^{64}Cu has fairly low maximum positron energy (0.66 MeV) and short positron range [20].

The positron-emitting diagnostic nuclides have a wide range of half-lives (10 min–to 12.7 h) and are reactor, cyclotron, or generator produced. ^{67}Cu is a therapeutic radionuclide that is currently produced on a high-energy accelerator [21].

Copper offers a relatively large number of radioactive isotopes that present relatively diverse nuclear properties and decay by positron (β^+) and/or beta minus (β^-)-emission. Additionally, the regional tissue distribution of all of these copper radioisotopes can be externally assessed with clinical gamma or positron imaging techniques [21].

Numerous developments have led to various applications for copper radioisotopes such as myocardial perfusion agents and hypoxia imaging agents, detection of multi-drug resistance, peptide targeted imaging and therapy, hypoxia-targeted therapy, radioimmunoimaging, and radioimmunotherapy.

4. Copper and its complexes – chemistry and types of ligands

The chemistry of copper in aqueous solution is restricted to two principal oxidation states (I and II). Copper(I) generally only exists in aqueous solution as a strong complex, since the free ion disproportionates to Cu^{2+} and copper metal (Cu^0). Cu^{3+} may be formed under certain conditions, but it is a powerful oxidant and is not a stable species in biochemical systems [21].

Metals exhibit preferences in binding geometry based on the number of valence d electrons and the number and type of coordinating ligands, as explained through the principles of ligand field theory (LFT). Based on LFT, differences in geometric preferences

for Cu(I) and Cu(II) are expected. Cu(I) is a d^{10} system and therefore does not experience, or have geometric preference based on, the ligand field stabilization energy (LFSE). It is often found coordinated by 2, 3, or 4 ligands in linear, trigonal, planar, or tetrahedral geometries. Cu(II) is a d^9 system that exhibits geometric preferences based in part on LFSE. It is often found coordinated by 4, 5, or 6 ligands, in square planar, square pyramidal, or axially distorted octahedral geometries, respectively, the latter being the result of Jahn–Teller distortion. Upon examination of many redox-active copper proteins that cycle between Cu(I) and Cu(II) states, we will see that the metal is predominately in tetrahedral geometry. Described as the entatic or rack state, the high energy Cu(I) geometry that is imposed on the oxidized form of the metal enhances its reactivity by increasing the redox potential [22].

Coordination chemistry has traditionally emphasized the design and synthesis of ligands that are able to control the electronic and steric properties of transition metal ions. In particular, chemists have been intrigued by the use of highly constrained ligands as a means of stabilizing unusual oxidation states or geometries. The study of "preorganized" ligands began in 1967 with Pedersen's landmark paper and has expanded to include macrocycles with nitrogen and sulfur atoms, cryptands, modified macrocycles with enhanced 3-D character, and tripodal ligands [23]. The chemical properties of the ligand set thus provide a choice of nitrogen or sulfur donor, neutral or anionic charge, pH-dependent or independent metal coordination, hydrophobic or hydrophilic character, the ability to bridge multiple metal centers, and different susceptibilities and consequences of oxidation of both the ligand and the metal [22].

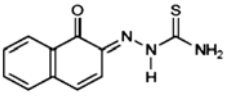
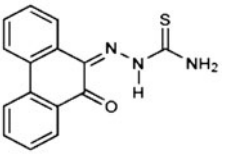
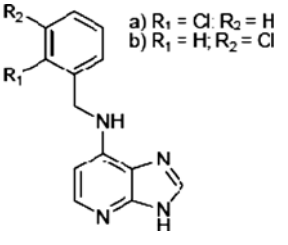
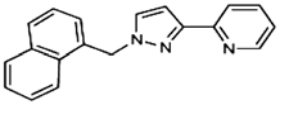
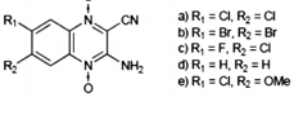
Biological copper is coordinated predominantly by just three ligand types: the side chains of histidine, cysteine, and methionine, with of course some exceptions. The arrangement of these components, however, is fascinating. The diversity provided by just these three ligands provides choices of nitrogen *versus* sulfur, neutral *versus* charged, hydrophilic *versus* hydrophobic, susceptibility to oxidation, and degree of pH-sensitivity [22].

The unique role of copper complexes and clusters can be seen in both physical and biological research and application. Particular attention is paid to the photochemical and photophysical properties of copper(I) complexes in light of the d^{10} electronic configuration, which diversifies their luminescent behavior [24].

Coordination polymers (also known as metal–organic frameworks) are a class of solid-state materials with infinite extended structures constructed from "connectors" (metals) and "linkers" (organic ligands) [25]. In recent years, some amendments have been proposed to complement the synthetic principle of "connectors and linkers", and one of the most familiar approaches is to introduce inorganic clusters or aggregates as "connectors" in place of single metal ions [26]. An important synthetic parameter for the preparation of molecular clusters and coordination polymers with interesting structures and properties is the appropriate selection of the ligand(s). A modern synthetic trend is the use of two or even three ligands in the reaction systems (combination of ligands or "ligand blends"). The loss of a degree of the synthetic control is more than compensated for by the diversity of structural types using the combination of ligands [27].

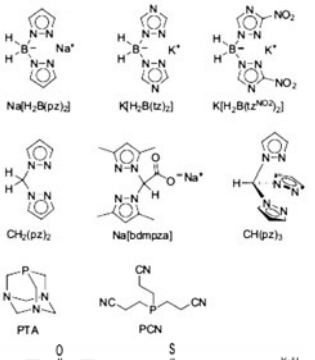
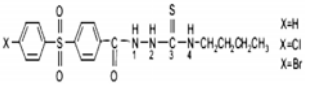
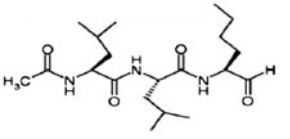
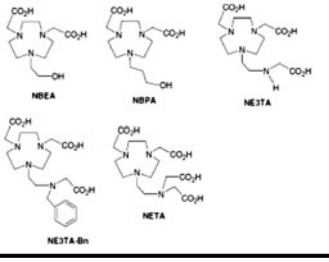
Some examples of important ligands for copper pharmaceuticals with their structure, description of their specific properties, and their use as Cu pharmaceuticals in oncology are given in table 2. Predominantly, the properties of ligands are characterized by antitumor and antiproliferative activity, cytotoxic selectivity or they bind to DNA by intercalation showing a marked DNA-cleavage activity.

Table 2. Some types of ligands.

Ligand	Structure	Properties of ligand	Usage
1,2-naphthaquinone thiosemi-carbazone		Significant antitumor properties; this cytotoxic action has been attributed to its topoisomerase II inhibitory activity	Against breast cancer cell line [28]
9,10-phenanthrenequinone thiosemi-carbazone		The copper complex exhibited maximum antiproliferative activity against the human breast cancer cell line, probably due to inhibition of steroid binding to the cognitive receptor or by preventing dimerization of the estrogen receptor	Against the human breast cancer cell line [29]
6-(2-chlorobenzylamino) purine **(a)** and 6-(3-chlorobenzylamino) purine **(b)**		The cytotoxic activity of the ligands strongly increased after formation of the Cu(II) complexes, which in turn showed potent activities against human malignant melanoma, human osteogenic sarcoma, and human breast adenocarcinoma	Against human malignant melanoma, human osteogenic sarcoma, and human breast adenocarcinoma [30]
2-[1-(naphthalen-1-ylmethyl)-1H-pyrazol-3-yl]pyridine		The ligand forms octahedral complex with copper. The compound had an extended aromatic p-system (like those shown by typical bidentate chelating ligands bipy and phen) and they bound to DNA by intercalation mode showing a marked DNA-cleavage activity	Superior cytotoxicity against human leukemia, human gastric cancer, and human mammary gland cancer cell lines [31]
AQCD derivatives		Bioreductive compounds, cytotoxic selectivity in hypoxia, insolubility, and low lipophilicity	Selective hypoxic cytotoxins [32]
$Na[H_2B(pz)_2]$ $K[H_2B(tz)_2]$ $K[H_2B(tz^{NO2})_2]$ $CH_2(pz)_2$ Na[bdmpza] $CH(pz)_3$		*In vitro* antitumor activity, increased lipophilic character of the ligand assembly	The cytotoxic properties against a panel of human tumor cell lines containing examples of breast, cervical,

(*Continued*)

Table 2. (*Continued*).

Ligand	Structure	Properties of ligand	Usage
PTA PCN			colon, lung cancer, leukemia, and melanoma [33]
N^1-[4-(4-X-phenyl sulfonyl)benzoyl]-N^4-butyl-thiosemicarbazide		Acylthiosemicarbazide contains oxygen, sulfur, and nitrogen as potential donor atoms and is liable to form deprotonated complexes by loss of hydrazinic proton *via* enolisation/ thioenolisation, because it might present a lot of tautomeric forms	The biochemical effects involved in the antitumor activity of ligands and some of newly synthesized complexes in human promyelocytic leukemia cells [34]
LLnL		Proteasome inhibitor of chymotrypsin; activity of LLnL is associated with induction of apoptosis in cancer cells	Induce apoptosis in intact tumor cells; (prostate cancer) [35]
polyaminocarboxylate-based chelators		Complexes with this ligands have an advantage of less protein interaction and a potentially more favorable *in vivo* tissue distribution	Use in iron depletion tumor therapy; the new ligands were radiolabeled with ^{64}Cu; colon cancer cell lines, potential chelates for radioimmuno-therapy [36]

5. Copper complexes in oncology

For thousands of years, metal complexes have played important and diverse roles in medicine [37]. From the biocide behaviors of copper complexes, to the long-standing application of gold complexes in Chinese and Arabic medicine, the unique and useful therapeutic benefits of metals have long been recognized and harnessed [38]. The therapeutic application of metal complexes in modern medicine was arguably initiated by the discovery of the anticancer properties of cisplatin [39]. In addition, to the continued clinical use of cisplatin against specific types of cancer, this discovery also inspired a new generation of effective, and in

some cases selective, metal-based cancer therapeutics, thereby demonstrating the potential of metal complexes as alternatives to classical "drug-like" small molecule inhibitors of human disease [40].

5.1. *Correlation between copper and tumor*

As we gain a better understanding of the factors affecting cancer etiology, we can design improved treatment strategies. Over the past three to four decades, there have been numerous successful efforts in recognizing important cellular proteins essential in cancer growth and therefore these proteins have been targeted for cancer treatment. However, studies have shown that targeting one or two proteins in the complex cancer cascade may not be sufficient in controlling and/or inhibiting cancer growth. Therefore, there is a need to examine features which are potentially involved in multiple facets of cancer development [41].

Several reports have shown that both serum and tumor copper levels in cancer patients are significantly elevated. A major percentage of these studies have focused on determining the concentrations of four important elements; copper, zinc, iron, and selenium. The studies showed that while zinc, iron, and selenium concentrations were significantly lower in cancer patients, the copper concentrations were almost always found to be either elevated or significantly elevated (up to 2–3-fold) compared to age-matched samples from normal tissue. Furthermore, it has been also shown that the Cu/Zn, Cu/Se, and Cu/Fe ratios are almost always higher in malignant patients compared to normal subjects [42, 43].

Serum copper level is raised in anemia, normal pregnancy, and during administration of oral contraceptives. Its level is also increased during infections due to the high activity of ceruloplasmin – one of the acute phase proteins. However, except from these indications, serum and tumor copper levels are significantly elevated in a variety of malignancies including breast, ovarian, gastric, lung, and leukemia [44].

Copper plays an important role in tumor angiogenesis, especially at its early stages. Copper seems to be necessary for endothelial cell activation as it stimulates their proliferation and migration. Several angiogenic factors, e.g. Vascular Endothelial Growth Factor (VEGF), basic fibroblast growth factor (bFGF), Angiogenin, Epidermal Growth Factor (EGF), tumor necrosis factor alpha (TNF-α), and interleukin (IL) 1 have been found to be copper activated. Activation factors bind to endothelial cells, "switch" them from G_0 into G_1 phase and force proliferation [45].

Probably, copper influences angiogenesis in a few ways. Not only does it activate endogenous angiogenic factors, but it also binds to several proteins (heparin, ceruloplasmin) which therefore gain angiogenic activity that manifests by stimulation of endothelial cells. It has also been shown that additional copper augments, by over four times, the angiogenin binding to calf pulmonary artery endothelial cells [18].

It can be summarized that various human cancer cells contain increased levels of copper, an essential cofactor for tumor angiogenesis. Without the copper-mediated tumor vascularization, tumors cannot grow or metastasize. It seems that tumor cells cannot grow more than 1–2 mm in diameter without angiogenesis, which supplies the tumor with oxygen and nutrients. It has been found that the cellular copper is not all protein-bound and can be stored in membranes. Therefore, a current strategy for cancer therapy is the inhibition of angiogenesis *via* copper control. Compounds developed for Wilson's disease, a copper storage disease, have recently been used to control the amount of copper found in tumor tissues as a method to prevent angiogenesis [46].

Copper can stimulate the production of cytokine, degradation of extracellular matrix, proliferation, and migration of endothelial cell. In recent years, the methods of controlling the growth of tumor by eliminating the copper in serum of human body have been developed [47].

5.2. *Copper complexes and their application in oncology diseases*

Knowledge about the complexion properties of copper (section 4) along with its distribution and action in organisms (homeostasis, section 2, tumor angiogenesis, section 5.1) is crucial in the development of pharmaceutically relevant/important copper complexes including their projection, synthesis, analysis, formulation in a proper dosage, and application in oncology. This section gives a brief view on the use of copper complexes in oncology. There is shown an overview of unlabeled (table 3) and labeled, i.e. radioactive (table 4) copper complexes used for therapy or diagnosis applied in cancer of soft and hard tissues from 2000 to the present. Soft tissue sarcomas can develop from soft tissues like fat, muscle, nerves, fibrous tissues, blood vessels, or deep skin tissues. Some of the hard tissues include the skeletal bones and cartilages. In addition, there are given structural formulas of copper complexes.

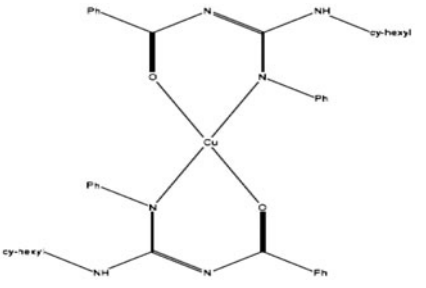
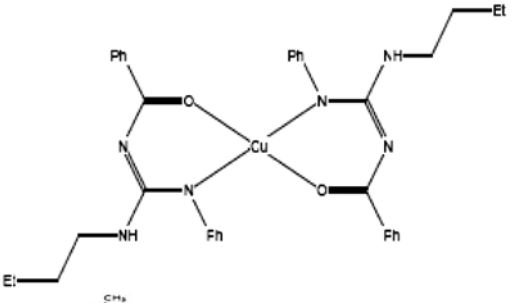
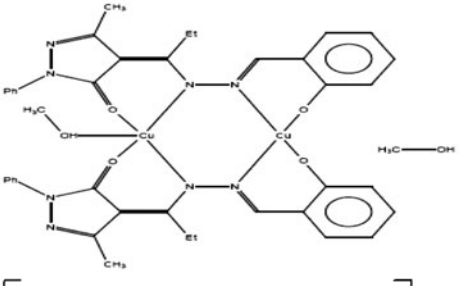
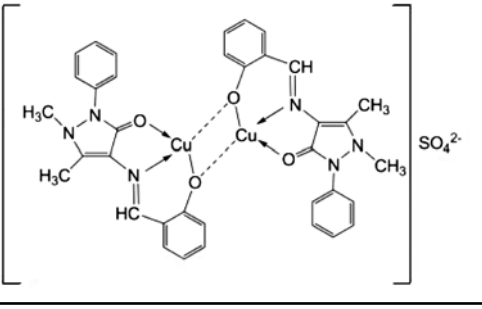
Most of the copper complexes were used either as anticancer agents or they were applied for the treatment of human promyelocytic leukemia cells and other applications for treatment of breast, lung, colon, kidney, pancreatic and prostate cancer, human squamous cervix carcinoma cells, endometrial and ovarian carcinoma, melanoma-skin, renal carcinoma, non-Hodgkin's lymphoma, human osteogenic sarcoma, malignant melanoma, and mammary gland cancer. A very significant application area presents copper complexes as tumor-imaging agents in PET for a higher selectivity for hypoxic or ischemic tissue.

It can be summarized, passing through the works briefly presented in tables 3 and 4, that in the group of unlabeled copper complexes, the copper complex with AMD (actinomycin-D) [Cu(AMD)(H_2O)] is very interesting and promising. In this complex, the antitumor activity of AMD is focused on DNA intercalation and this complex is used as anticancer drug widely applied in therapy. On the other hand, the copper complexes with the ligands DOTA, EDTA, DTPA, TETA, and NOTA are most widely applied among the labeled copper complexes. It is due to their good properties for both PET imaging and therapy as well.

It was demonstrated that low concentration of D-pen in the presence copper resulted in a concentration-dependent H_2O_2-mediated cytotoxicity in breast cancer. D-pen in the presence of copper also was shown to generate concentration-dependent cellular ROS and to decrease cellular reduced thiol content in cancer cells. D-pen has effective ROS-generating ability in the presence of copper. Since copper levels are significantly elevated in the serum and tumor tissue in a variety of malignancies, these findings provide a novel and exciting opportunity to exploit D-pen as an anticancer agent having both antiangiogenic and cytotoxic mechanism of action [61].

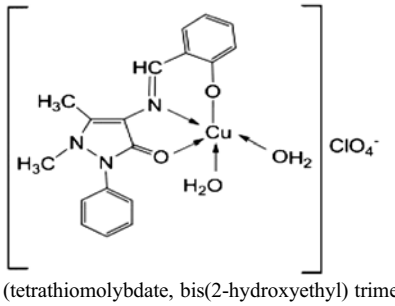
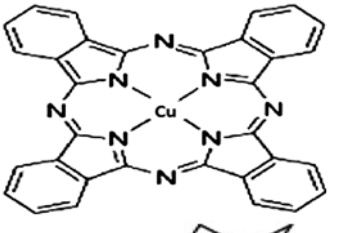
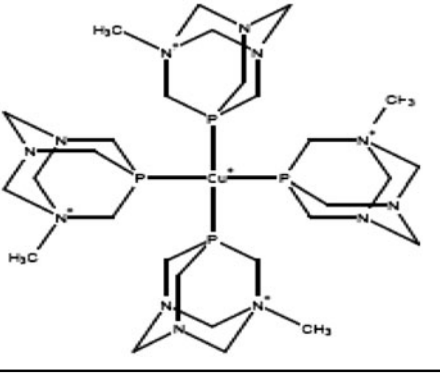
Copper complexes of salicylaldehyde pyrazole hydrazone (SPH) could induce apoptosis in lung cancer cells. It has been shown that the SPHs themselves did not cause apoptosis in the cells. It seems that they have to form a complex with copper ions to carry out the apoptosis-promoting effect, consistent with the situation in some reported SBHs in which the copper complexes exhibit stronger anti-proliferative effect [79]. The reason for this phenomenon could be due to the alteration of conformation and electric property as well as hydrophilicity in the process of copper–SPH complex formation, which subsequently enables the complexes to target key regulators of apoptosis. Integrin β4 plays important roles in the

Table 3. Unlabeled copper complexes and their application in oncology.

Chemical formula of copper complexes	Structure of the complexes	Application in oncology
Bis(N-phenyl-N′-cyclohexyl-N″-benzoyl-guanidinato) copper(II)		Breast cancer, renal cancer, lung cancer, ovarian cancer, melanoma, and colon cancer [48]
Bis(N-phenyl-N′-(n-butyl)-N″-benzoyl-guanidinato) copper(II)		Renal cancer, breast cancer, lung cancer, ovarian cancer, melanoma, and colon cancer [48]
N-(1-phenyl-3-methyl-4-propyl-pyrazolone-5)-salicylidene hydrazone (H_2L) and its copper(II) complex $[Cu_2L_2CH_3OH].2CH_3OH$		Used as anticancer agent [49]
$[Cu_2(C_{18}H_{16}N_3O_2)_2]\ SO_4$		Human promyelocytic leukemia cells, antitumor activity [50]

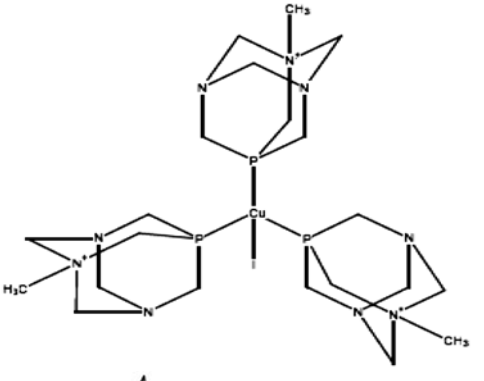
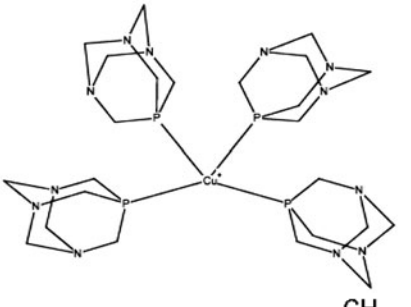
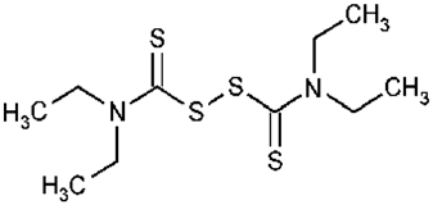
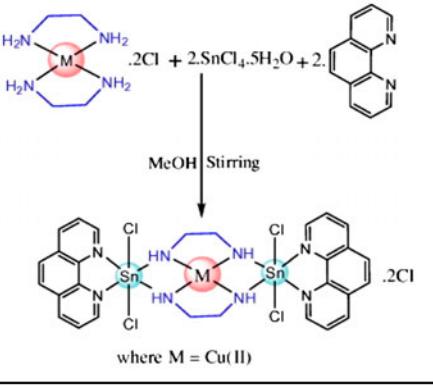
(*Continued*)

Table 3. (*Continued*).

Chemical formula of copper complexes	Structure of the complexes	Application in oncology
$[Cu(C_{18}H_{16}\text{-}N_3O_2)(H_2O)_2]ClO_4$		Human promyelocytic leukemia cells, antitumor activity [50]
ATN-224 and TM both bind to Cu^{2+} (a)	(tetrathiomolybdate, bis(2-hydroxyethyl) trimethyl - ammonium; choline tetrathiomolybdate) is the bis-choline salt of TM, a novel copper chelator	Anti-tumor and anti-angiogenic effects against a murine lung cancer xenograft and a murine inflammatory breast cancer model [51]
Copper-PC		Is involved in the etiology of several chronic diseases, including cardiovascular disease, diabetes, cancer, and neurodegenerative disorders [52]
$[Cu(mPTA)_4][(CF_3SO_3)_2(BF_4)_3] \cdot 0.25H_2O$		Ovarian cancer cells, human squamous cervix carcinoma cells, non-small lung carcinoma cells, more effective, and less-toxic anticancer drug [53]

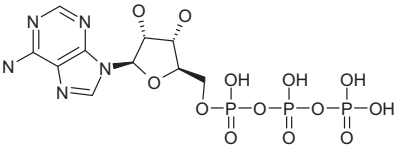
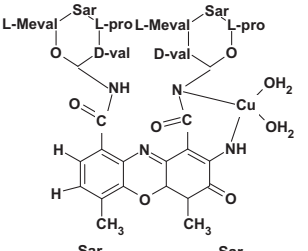
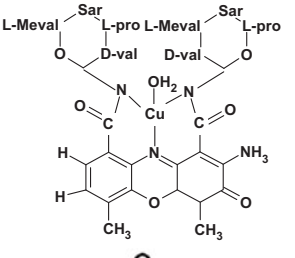
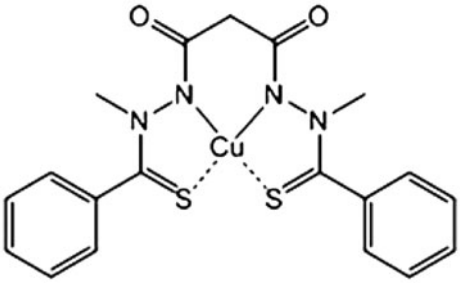
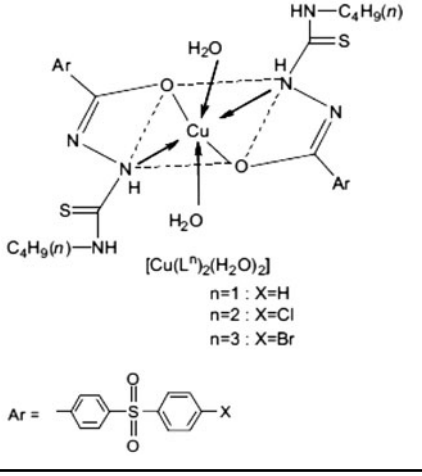
(*Continued*)

Table 3. (*Continued*).

Chemical formula of copper complexes	Structure of the complexes	Application in oncology
$[Cu(mPTA)_3I][I]_3 \cdot H_2O$		Ovarian cancer cells, human squamous cervix carcinoma cells, non-small lung carcinoma cells, more effective, and less-toxic anticancer drug [53]
$[CuPTA_4]\ BF_4 \cdot 6H_2O$		Ovarian cancer cells, human squamous cervix carcinoma cells, non-small lung carcinoma cells, more effective, and less-toxic anticancer drug [53]
DS/Cu complex (a)		DS may specifically target cancer and spare normal tissues, may be used in clinic to improve the therapeutic effect in breast and colon cancer patients [54]
[Cu (4,7-dimethyl-1,10-phenanthroline)(glycine) NO_3] or casiopeina II (a)	–	On the murine leukemia and human ovarian carcinoma cells [55]
$[Cu(en)_2(phen)_2Sn_2Cl_4]\ Cl_2$		The cell lines used for *in vitro* antitumor screening, kidney, breast, cervix, lung, colon, and pancreatic Less harmful and more prone to antiproliferative activity against tumors [56]

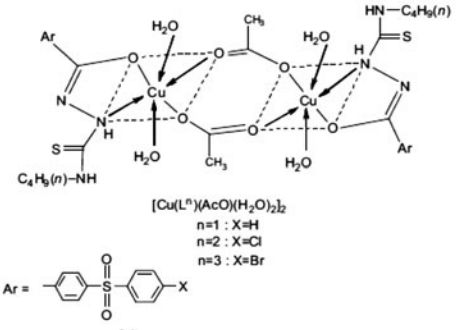
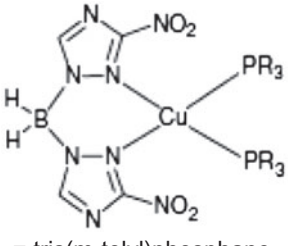
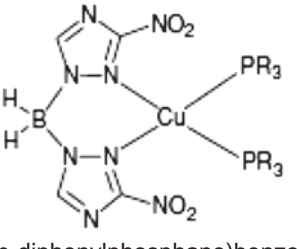
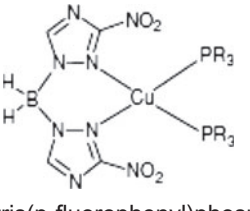
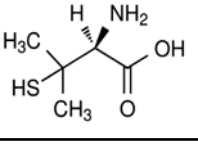
(*Continued*)

Table 3. (*Continued*).

Chemical formula of copper complexes	Structure of the complexes	Application in oncology
Copper-transporting P-type adenosine triphosphatase (ATP7B) (a)		Endometrial and ovarian carcinoma [57]
$[Cu(H_2AMD)(H_2O)_2]^+$		Anti-tumor activity of AMD, which is focused on DNA intercalation, anticancer drug widely applied in therapy [58]
$[Cu(AMD)(H_2O)]$		Anti-tumor activity of AMD, which is focused on DNA intercalation, anticancer drug widely applied in therapy [58]
Elesclomol – Cu		Selective induction of oxidative stress in cancer cell mitochondria, represents an approach distinct from that of chemotherapy or kinase inhibition for therapeutic intervention in human malignancies, implications for cancer therapy [59]
N^1-[4-(4-X-phenyl sulfonyl) benzoyl]-N^4-butyl-thiosemicarbazide, X = H, Cl, Br with Cu^{2+} $[Cu(L^3)_2(H_2O)_2]$ $(C_{36}H_{42}Br_2CuN_6O_8S_4)$ $[Cu(L^2)_2(H_2O)_2]$ $(C_{36}H_{42}Cl_2CuN_6O_8S_4)$ $[Cu(L^2)_2(H_2O)_2]$ $(C_{36}H_{44}CuN_6O_8S_4)$ $[Cu(L^1)_2(H_2O)_2]$ $(C_{36}H_{44}CuN_6O_8S_4)$		Effects of these complexes on the growth of human promyelocytic leukemia cells and antibacterial activity [34]

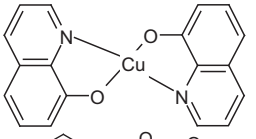
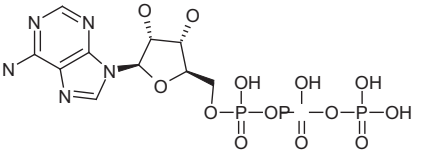
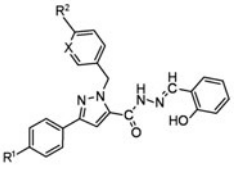
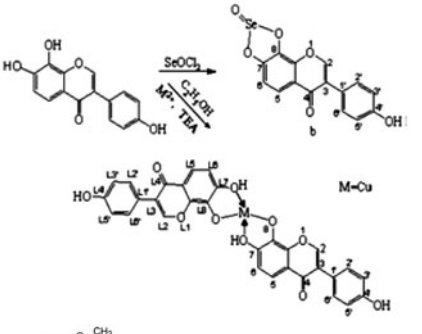
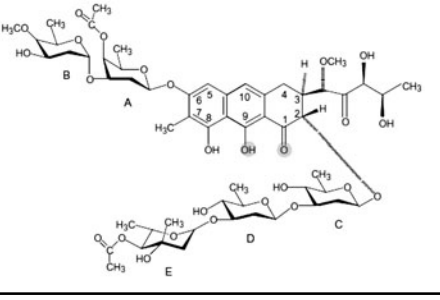
(*Continued*)

Table 3. (*Continued*).

Chemical formula of copper complexes	Structure of the complexes	Application in oncology
$[Cu_2(L^1)_2(AcO)_2(H_2O)_4]$ ($C_{40}H_{54}Cu_2N_6O_{14}S_4$) $[Cu_2(L^2)_2(AcO)_2(H_2O)_4]$ ($C_{40}H_{52}Cl_2Cu_2N_6O_{14}S_4$) $[Cu_2(L^3)_2(AcO)_2(H_2O)_4]$ ($C_{40}H_{52}Br_2Cu_2N_6O_{14}S_4$)		Human promyelocytic leukemia cells [34]
$[H_2B(tz^{NO2})_2]Cu[P(m\text{-}tolyl)_3]_2$ (the first nitro-substituted heteroscorpionate ligand)	 PR_3 = tris(m-tolyl)phosphane	Cytotoxic properties against a panel of five human tumor cell lines containing examples of ovarian, cervix and lung cancer, melanoma, and leukemia [60]
$[H_2B(tz^{NO2})_2]Cu[P(C_6H_5)_2(p\text{-}C_6H_4COOH)]_2$	 PR_3 = (p-diphenylphosphane)benzoic acid	Cytotoxic properties against a panel of five human tumor cell lines containing examples of ovarian, cervix and lung cancer, melanoma, and leukemia [60]
$[H_2B(tz^{NO2})_2]Cu[P(p\text{-}C_6H_4F)_3]_2$	 PR_3 = tris(p-fluorophenyl)phosphane	Cytotoxic properties against a panel of five human tumor cell lines containing examples of ovarian, cervix and lung cancer, melanoma, and leukemia [60]
D-pen plus cupric sulfate and H_2O_2 (a)		Complex is cytotoxic to human leukemia and breast cancer cells [61]

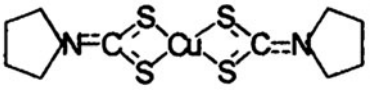
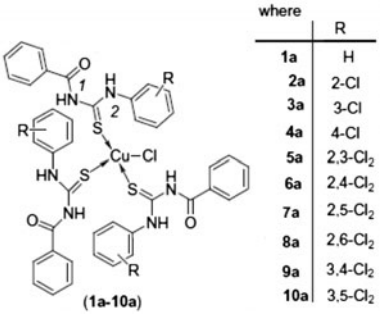
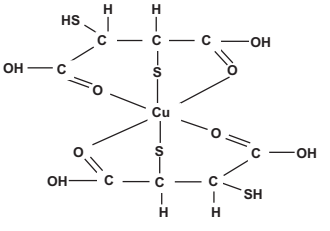
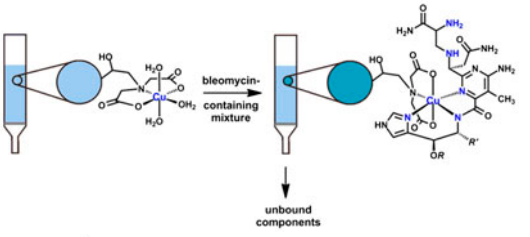
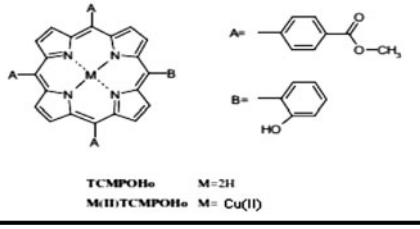
(*Continued*)

Table 3. (*Continued*).

Chemical formula of copper complexes	Structure of the complexes	Application in oncology
$[Cu(8\text{-}OHQ)_2]$		Induce apoptosis in intact tumor cells (prostate cancer) [35]
Copper-transporting P-type adenosine triphosphatase (ATP7B) (a)		The induction of ATP7B gene was observed by exposure to cisplatin in the human prostate carcinoma cell line [62]
(Cu-SPHs) = copper complexes of SPH derivatives (a)	 Compound R^1 R^2 X 4 H Cl N 8 Cl Cl N 12 OCH_3 Cl N 15 H H C 16 H *t*-Bu C 17 Cl H C 18 Cl *t*-Bu C 19 OCH_3 H C 20 OCH_3 *t*-Bu C	Copper–SPH complexes could induce apoptosis in lung cancer cells, and suggested that Cu-16 might be a potential selective therapeutic drug for lung cancer, anti-proliferative and anticancer activity, non-small cell lung carcinoma, lung adenocarcinoma, and squamous cell carcinoma [63]
4′,7,8-Trihydroxy-isoflavone with transition copper		New chemotherapy agent, against five different cancer cell lines: human breast carcinoma cell line, human uterine cervix cancer cell line, human liver cancer cell line, human colon carcinoma cell line, and human lung carcinoma cell line [64]
$Cu^{II}(Chro)_2$ DNA-$Cu^{II}(Chro)_2$ (DNA = Synthetic hairpin DNA, TTGGCCAATG TTTGGCCAA) (a)		The cytotoxicity of these complexes in the human hepatoblastoma cell line cancer cell line, anti-cancer metallo-antibiotics that contain transition metal ions [65]

(*Continued*)

Table 3. (*Continued*).

Chemical formula of copper complexes	Structure of the complexes	Application in oncology
$(PyDT)_2Cu$		Copper complex-induced proteasomal inhibition and apoptosis, which should have a clinical significance [66]
[CuCl(PBT)3][1a] [CuCl(2-CPBT)3][2a] [CuCl(3-CPBT)3][3a] [CuCl(4-CPBT)3][4a] [CuCl(2,3-DCPBT)3][5a] [CuCl(2,4-DCPBT)3][6a] [CuCl(2,5-DCPBT)3][7a] [CuCl(2,6-DCPBT)3][8a] [CuCl(3,4-DCPBT)3][9a] [CuCl(3,5-DCPBT)3] [10a]		The complexes have been screened for their *in vitro* cytotoxic activity in seven human cell lines carcinomas Renal, Breast, Lung, Ovarian, Melanoma-Skin, Colon [67]
Cu(II)[DMSA]$_2$		Adept for new radiopharmaceutical, DMSA labeled with ^{99m}Tc is used for renal imaging and is a useful agent for imaging of thyroid carcinoma, head/neck tumors and brain, liver and skeletal metastases [68]
[Cu(IDA)(bleomycin)]		High-value therapeutic anticancer agent [69]
[Cu(II)TCMPOHo]		Cytotoxic activity in human Caucasian histiocytic lymphoma [70]

Note: (a): X-ray structure of Cu complex is unknown.

signaling networks that drive tumor progression [80] and it was expressed at a high level in lung carcinoma cells. Integrin β4 appears to play dual roles in cancer development, either as a tumor suppressor or a promoter, depending on the status of p53, since it stimulated apoptosis in p53 wild-type cancer cells, but promoted survival in p53 mutant cells. Copper–SPH complexes might be used as a useful tool to decipher the molecular mechanism of integrin β4 in regulating apoptosis in lung cancer or in tumors in general [63].

Certain derivatives of quinoline in combination with $CuCl_2$ functioned similar to each other but quite different from complexes of known anti-copper drugs and copper. These results support the mechanism of the current anti-copper drugs as strong chelators of copper, rendering the copper ion unavailable for use by the cell. However, other ligands may be capable of binding copper in such a manner that it would possess proteasome inhibitory properties. It was tested whether or not the copper ligand 8-OHQ could interact with cellular copper and result in proteasome inhibition and apoptosis induction. Indeed, prostate cancer cells grown in copper-enriched conditions (to generate cells bearing elevated copper as found in tumor tissues) are sensitive to treatment with ligand alone. These data support the idea that copper in the tumor local cellular environment could be converted to a cell death inducer through the mechanism of proteasome inhibition by exposure to a copper-binding ligand. It is assumed that the function of a copper ligand is to shield the copper from metabolic use by the cell, to aid delivery of the copper to the proteasome, and to increase the effectiveness of the copper-mediated proteasome-inhibitory activity [35]. A unique feature of cancer cells is to accumulate high concentrations of copper [81]. Cellular concentrations of copper in tumor cells and tissues should be high enough to promote complex formation. Tumors could be treated with copper ligands alone that would be non-toxic to cells, but could bind to tumor cellular copper, forming potent proteasome inhibitors. It is possible that the affinity of a copper ligand for copper bearing cancer cells and the transient nature of the proteasome inhibition might protect normal cells from toxicity [35].

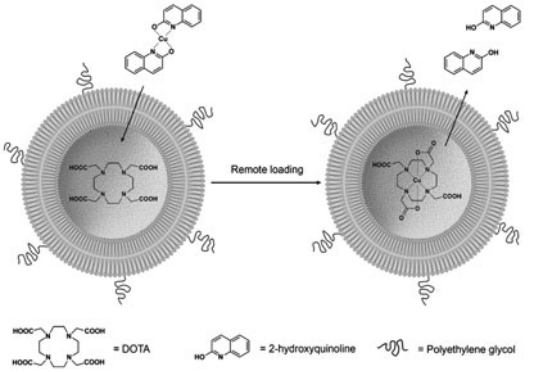
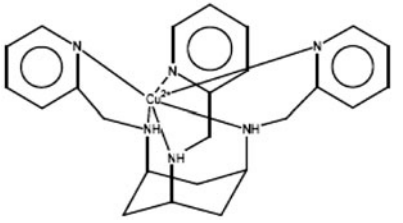
5.3. *Some selected ligands of copper complexes used as anticancer/antitumor agents*

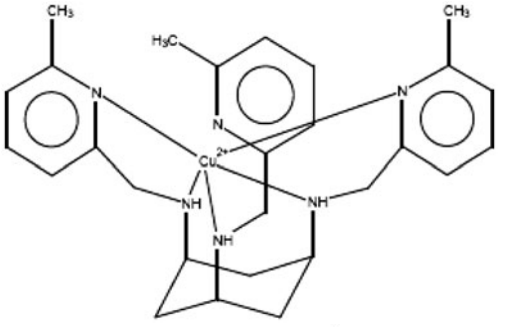
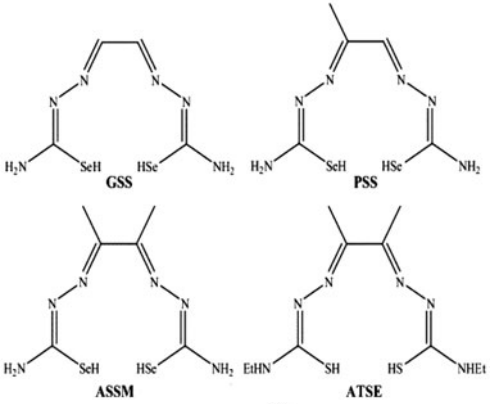
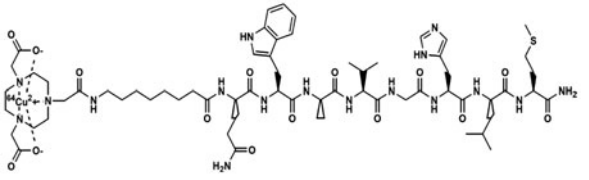
5.3.1. Actinomycin-D. Actinomycin was extracted in ether from the culture media of the organism *actinomyces*, now designated *Streptomyces antibioticus*. The substance was crystallized in alcohol as a red pigment [82]. Actinomycin-D (AMD) was isolated as one of the mixture of three different actinomycins from *Streptomyces chrysomallus* [83].

AMD is a chromopeptide composed of heterotricyclic chromophore (which absorbs in the visible range), and two pentapeptide lactone rings. The chromophore, 2-amino-4,6-dimethylphenoxazin-3-one-1,9-dicarboxylic acid, called actinocin, is responsible for the reddish color of AMD [84].

In a major structural elucidation, Sobell *et al.* [85] confirmed by X-ray crystallography that AMD intercalates into DNA from the single crystal of the complex with deoxyguanosine base. The structure of the complex revealed the twofold symmetry of the molecule of actinomycin; this finding indicates that two equivalent binding sites are available to deoxyguanosine during the complex formation. The model supports the data suggesting intercalation as well as hydrogen-bonded guanine recognition. The ability of AMD to intercalate DNA and to inhibit DNA-dependent RNA synthesis is now widely accepted as AMD's major mode of action. Because of AMD's specificity in inhibiting RNA synthesis, AMD has become an agent used routinely in biology laboratories [86].

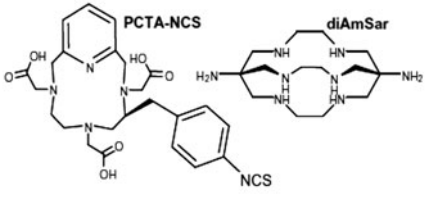
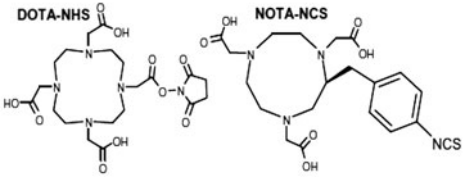
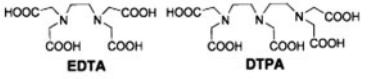
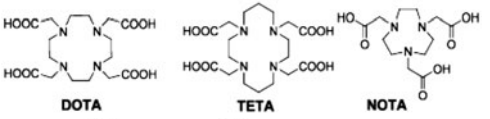
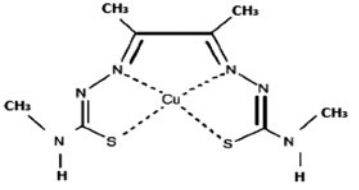
Table 4. Labeled copper complexes and their application in oncology.

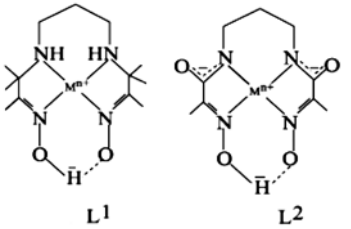
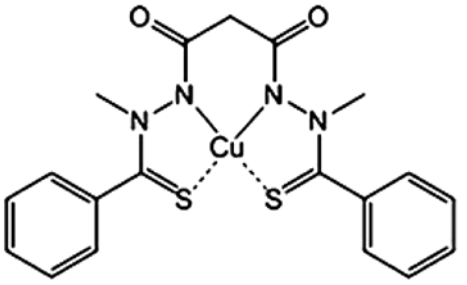
Chemical formula of copper complexes	Structure of the copper complexes	Application in oncology
^{64}Cu-DOTA using 2-hydroxyquinoline		Imaging tool for assessing tumor response to new molecular entities or targeted nanomedicine such as cytostatic agents that have predominantly anti-angiogenic or anti-proliferative effects, as a cancer diagnostic agent; application in bearing colon adenocarcinoma [20]
[$^{67,\ 64}$Cu(tachpyr)]$^{2+}$		An attractive candidate for developing new Cu(II) radiopharmaceuticals for diagnosis and therapy [71]

[$^{67,\ 64}$Cu(tach-6-Me-pyr)]$^{2+}$		An attractive candidate for developing new Cu(II) radiopharmaceuticals for diagnosis and therapy [71]
[^{64}Cu-ASSM] [^{64}Cu-ATSE] [^{64}Cu-GSS] [^{64}Cu-PSS] (a)		Tumor-imaging agents in PET for a higher selectivity for hypoxic or ischemic tissue [72]
[^{64}Cu-NO2A-8-Aoc-BBN(7–14)NH_2] (a)		Imaging agent for human breast cancer tumors [73]
[^{64}Cu]-NOTA-RNA		

(*Continued*)

Table 4. (*Continued*).

Chemical formula of copper complexes	Structure of the copper complexes	Application in oncology
[^{64}Cu]-PCTA-RNA added *via* the p-Bn-SCN linker group		^{64}Cu-labeled RNA aptamers to prostate cancer cells, RNA-based PET molecular-imaging agents [74]
		
^{67}Cu-2IT-BAT-Lym-1	–	For non-Hodgkin's lymphoma radioimmuno-therapy [75]
[^{64}Cu]-DOTA-RNA added *via* the NHS ester		
[^{64}Cu]-diAmSar-RNA (a)		
[^{64}Cu]-EDTA [^{64}Cu]-DTPA [^{64}Cu]-DOTA [^{64}Cu]-TETA [^{64}Cu]-NOTA (a)		For PET imaging (β+) and therapy (β+ and β−) [76]

[^{64}Cu] ATSM	 L^1 L^2	Potential use of this radiotracer for imaging in the field of oncology (tumor hypoxia) [77]
^{64}Cu[CuL^{1}H$_{-1}$] ^{62}Cu[CuL^{2}H$_{-1}$] ^{60}Cu[CuL^{2}H$_{-1}$] **L^1** = 3,3,9,9-tetramethyl-4,8-diazaundecane-2,10-dione dioxime **L^2** = N, N′-bis(2-hydroxyiminopropionyl) propane-1,3-diamine		^{64}Cu[CuL^{1}H$_{-1}$] –chemotherapeutic agent, ^{62}Cu[CuL^{2}H$_{-1}$] and ^{60}Cu[CuL^{2}H$_{-1}$] - great importance in the imaging of the renal system [78]

Note: (a): X-ray structure of Cu complex is unknown.

In 1979 Ross *et al.* [87] determined by alkaline elution that AMD, like other intercalating agents, caused protein-associated strand breaks. They proposed that an "intercalation-induced distortion of the DNA helix leads to strand scission by a nuclease which becomes bound to one terminus of the break so as to form a DNA-protein crosslink." Since that publication, Wang [88] isolated the proposed nuclease and identified it as topoisomerase II. Topoisomerase II is a DNA gyrase that is responsible for altering the topology of DNA during replication, to allow polymerases to act on the DNA. The ability of intercalating agents like AMD to form a tertiary complex with topoisomerase II and the DNA may be an alternate cause of their cytotoxicity.

AMD is used in the treatment of different oncological diseases as a desirable chemotherapeutic agent, in combination with drugs such as etoposide, vinblastin, vincristine, doxorubicin, cyclophosphamide, and bleomycin (BLM). In this review, two copper complexes with AMD in table 3 ($[Cu(H_2AMD)(H_2O)_2]^+$ and $[Cu(AMD)(H_2O)]$) are introduced and are used as anticancer drug with wide application in therapy.

5.3.2. Bleomycin (BLM). The BLMs are a family of structurally related glycopeptides-derived antibiotics [89]. They have demonstrated efficacy in the clinic against several types of neoplasms, notably squamous cell carcinomas and malignant lymphomas, and are now used routinely as antitumor agents [90]. The earliest members of this group of antibiotics were the phleomycins (PLMs), which were isolated from *Streptomyces verticillus* by Umezawa and co-workers [91] prior to the discovery of the BLMs [92]. In spite of the seemingly small structural difference between these types of compounds, isolation and characterization of the PLMs have provided important insights into the anticancer activity and mechanism of action of this entire group of antibiotics, as the PLMs differ from the BMLs in their behavior in certain respects.

Although BLM is believed to exert its antitumor effects as part of a metallo-BLM complex [93], early studies indicated that direct administration of a Cu chelate of BLM caused local necrosis at the site of injection. Accordingly, subsequent studies employed a metal-free solution of BLM, and this is the way in which BLM is administered clinically.

Although BLM is believed to function as an antitumor agent by virtue of its ability to damage chromosomal DNA, little information is available concerning the extent to which BLM enters cells or the actual mode of drug uptake [94].

BLM is employed in combination with other antitumor agents for the treatment of testicular carcinomas, germ cellovarian cancers, and non-Hodgkin's lymphomas [95]. These protocols are often curative. BLM is also used as a single agent and in combination chemotherapy for the treatment of squamous cell carcinomas of the head and neck, skin, and cervix [96]. In this review, copper complex with BLM in table 3 ([Cu(IDA)(bleomycin)]) is introduced and is used as high-value therapeutic anticancer agent.

6. Conclusions

Monitoring a huge amount (thousands) of the newly prepared copper complexes from 2000 up to now (2013), it has been found out that only a very little part of them have been considered for a medicinal use (research or clinical). This review summarized the most important from them aimed to oncology.

This review summarized copper complexes used for therapy or diagnosis of various diseases in oncology from 2000 to the present. Together, it was found 96 copper complexes, 69 unlabeled, and 27 labeled copper complexes applied in cancer of soft and hard tissues. They are mostly monomeric and dimeric molecules primarily with N and O donating atoms. The ligands which directly coordinated to Cu(II) are mono-, bi-, ter-, and even tetra-dentate, from which heterobidentate (O,N) is the most frequent. In the Cu(II) complexes, Cu(II) ions are mostly in a square planar arrangement. The complexes have specific effect, for example, they could induce apoptosis in cancer cells or they are antiproliferative anticancer agents with cytotoxic selectivity.

A unique feature of cancer cells is to accumulate high concentrations of copper [97]. We believe that a strategy could be developed to bind this copper with specific ligands and form an anticancer agent within tumor tissues/cells. Cellular concentrations of copper in tumor cells and tissues should be high enough to promote complex formation. Tumors could be treated with copper ligands alone that would be non-toxic to cells, but could bind to tumor cellular copper, forming potent proteasome inhibitors. It is possible that the affinity of a copper ligand for copper-bearing cancer cells and the transient nature of the proteasome inhibition might protect normal cells from toxicity [98].

A thorough understanding of the anti-proliferative mechanism of copper chelators will require a comprehensive identification of the copper proteome and a deeper understanding of the mechanisms of copper homeostasis and regulation. The copper complexes are promising for extension scale of cancerostatic drugs.

Abbreviations

AQCD	3-aminoquinoxaline-2-carbonitrile N1,N4-dioxide
AMD	actinomycin-D
ASSM	diacetyl-bis(selenosemi-carbazone)
ATSE	diacetyl-bis(N4-ethylthiosemi-carbazone
ATSM	diacetylbis(N4-methyl-thiosemi carbazone)
BAT	6-[p-(bromoacetamido) benzyl]-TETA
BBN	Bombesin
bFGF	basic fibroblast growth factor
BLM	Bleomycin
$CH(pz)_3$	k_3-N,N,N tris(pyrazol-1-yl) methane
	$CH_2(pz)_2$bis (pyrazol-1-yl) methane
Ctr1	Cu transporter 1
CPBT	1-Chlorophenyl-3-benzoylthiourea
Cu/Zn-SOD	Cu/Zn-superoxide dismutase
[Cu(II)TCMPOHo]	Cu(II)-5-(2-hydroxyphenyl)
	10,15,20etris(4-carboxymethylphenyl) porphyrin
DCPBT	1-Dichlorophenyl-3-benzoylthiourea
diAmSar	3,6,10,13,16,19-hexa azabicyclo[6.6.6]icosane-1,8-diamine
DMSA	meso-2,3-dimercaptosuccinic acid
DMT1	divalent metal transporter 1
DNA	Deoxyribonucleic acid
DOTA	1,4,7,10-tetraazadodecane-N,N′,N″,N‴-tetraacetic acid
D-pen	D-penicillamine

DS	Disulfiram
DTPA	diethylene triamine pentaacetic acid
EGF	Epidermal Growth Factor
EDTA	ethylenediaminetetra-acetic acid
GSS	glyoxal-bis(selenosemi-carbazone)
$[H_2B(tzNO_2)_2]$-	dihydridobis(3-nitro-1,2,4-triazolyl)borate
Chro	Chromomycin A3
IDA	iminodiacetate
IL	interleukin
$K[H_2B(tz)_2]$	dihydrobis (triazolyl) borate potassium salt
$K[H_2B(tzNO_2)_2]$	dihydrobis (3-nitro-1,2,4-triazolyl) borate potassium salt
LFSE	ligand field stabilization energy
LFT	ligand field theory
LLnL	N-acetyl-L-leucinyl-L-leucinal-L-norleucinal
Lym-1	Antilymphoma monoclonal antibody
mPTA	N-methylated 1,3,5-triaza-7-phosphaad amantane
MTs	metallothioneins
Na[bdmpza]	k3-N,N,O bis(3,5-dimethylpyrazol-1-yl) acetate sodium salt
$Na[H_2B(pz)_2]$	bidentate k_2-N,N dihydrobis (pyrazolyl) borate sodium salt
NOTA	1,4,7-triazacyclononane-1,4,7-triacetic acid
NO	1,4,7-triazacyclononane-1,4-diacetate
PBT	1-Phenyl-3-benzoylthiourea
PC	phthalocyanine
PCN	tris(cyanoethyl) phosphine
PCTA	3,6,9,15-tetraazabicyclo [9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid
PET	positron emission tomography
phen	1,10-phenanthroline
PLMs	phleomycins
PSS	pyruv- aldehyde-bis(selenosemi- carbazone)
PTA	1,3,5-triaza-7-phosphaadamantane
PyDT	pyrrolidine dithiocarbamate
ROS	reactive oxygen species
RNA	Ribonucleic acid
SPHs	salicylaldehyde pyrazole hydrazone derivatives
tachpyr	N,N_,N_-tris(2-pyridylmethyl)-1,3,5-cis,cis-triaminocyclohexane
tach-6-Me-pyr	N,N_,N_-tris(6-methyl-2-pyridylmethyl)-1,3,5-cis,cis-triamino-cyclohexane
TETA	1,4,8,11-tetraazacyclotetra-decane-1,4,8,11-tetraacetic acid
TM	tetrathiomolybdate
TNF-α	tumor necrosis factor alpha
VEGF	Vascular Endothelial Growth Factor
2IT	2-iminothiolane
(7–14)NH2	8-Aminooctanoic Acid)-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH2
8-OHQ	bis-8-hydroxyquinoline

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