STUDIA UNIVERSITATIS MOLDAVIAE, 2021, nr.6(146)
Seria "Științe reale și ale naturii" ISSN 1814-3237 ISSN online 1857-498X p.132-140

CZU: 57.085:615.277 **DOI: https://doi.org/10.5281/zenodo.5702096**

SOME INORGANIC AND ORGANIC BIOLOGICAL AGENTS

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Based on the known methods and their modifications, 3 compounds were synthesized: 2-formylpyridine *N*(4)-phenylthiosemicarbazone**,** complex copper(II) [Cu(L)Cl] with 2-formylpyridine *N*(4)-phenylthiosemicarbazone ligand, copper(II) mixed-ligand complex chloro(*N*-phenyl-*N*'-[(pyridin-2-yl)methylidene]carbamohydrazonothioato)(4-aminobenzene-1-sulfonamide)copper [Cu(Str)(L)Cl]. The antiproliferative properties of these compounds towards cancer cell lines MeW-164 and HeLa have been investigated. The tested compounds demonstrated antiproliferative and selective activity towards cancer cells. The tested compounds inhibit the activity of LOX. Direct toxic evaluation of compounds was performed by *Daphnia magna* bioassay. It was found that the tested compounds have a lower toxicity than DOXO. Theoretical pharmacokinetics (Lipinski's rule, PSA) of CMT-22, CMT-67 and CMT-68 supports further *in vivo* studies.

Keywords: *anticancer compound, DNA fragmentation, apoptosis, antilipoxygenase activity, Lipinski's rule.*

AGENȚI BIOLOGICI ORGANICI ȘI ANORGANICI

În baza metodelor cunoscute și a modificărilor lor, au fost sintetizați 3 compuși: N(4)-feniltiosemicarbazona 2-formilpiridinei (N-fenil-2-(piridin-2-ilmetiliden)hidrazincarbotioamida, CMT-22, HL), complexul de cupru(II) [Cu(L)Cl] cu ligandul CMT-22 (cloro(N-fenil-N'-[(piridin-2-il)metiliden]carbamohidrazonotioato)cupru, CMT-67), complexul de cupru(II) cu liganzi micști [Cu(Str)(L)Cl] cu CMT-22 și 4-aminobenzensulfonamidă (Str) (cloro(N-fenil-N'-[(piridin-2-il) metiliden]carbamohidrazonotioato)(4-aminobenzen-1-sulfonamidă)cupru, CMT-68). Au fost investigate proprietățile antiproliferative ale acestor compuși față de liniile celulare canceroase MeW-164 și HeLa. Compușii testați au demonstrat o activitate antiproliferativă și selectivă față de celulele canceroase. Compușii testați inhibă activitatea LOX. Evaluarea toxică directă a compușilor a fost efectuată prin testul biologic *Daphnia magna.* A fost constatată toxicitatea mai mică a compușilor testați decât DOXO. Farmacocineticele teoretice (regula lui Lipinski, PSA) ale CMT-22, CMT-67 și CMT-68 sunt susținute prin studii suplimentare *in vivo*.

Cuvinte-cheie: *compus anticancer, fragmentarea ADN-ului, apoptoză, activitate antilipoxigenază, regula lui Lipinski.*

Introduction

According to a recent report by the WHO, there are now more than 10 million cases of cancer per year worldwide [1]. Cancer refers to a diversity of diseases, characterized by the uncontrolled proliferation of cells. The continuous proliferation of cancer cells develops into tumour tissues and may spread across to other organs. It is known that a wide variety of genes are involved in the development of tumours and many cell processes are deregulated, including mechanisms for controlling cell proliferation, DNA repair, chromosome stability, cell-cell interactions, cell-matrix interactions, angiogenesis, cell aging and apoptosis. In this regard, it is necessary to take into account the basic cellular processes for correct prescriptions of anticancer drugs and understanding their mechanism of action.

High systemic toxicity and drug resistance remain a major challenge for modern medicine in the management of cancer despite the significant progress made in the anticancer therapy. Chemotherapy can produce severe side effects caused by its cytotoxic effect on normal cells. This limits their use and it is an indication to reduce the drug dose, interrupt and even cease the treatment. Therefore, it is important that the anticancer drugs exert antiproliferative and cytotoxic activity in tumour cells without affecting normal tissues, so the principal need in the chemoprevention of cancer remains the discovery of new agents that are effective and safe.

Antineoplastic agents are divided into cytotoxic and cytostatic. Doxorubicin, cisplatin, fluorouracil, hydroxyurea, and cyclophosphamide are the most known among the cytostatic drugs. Doxorubicin (DOXO), a front-line drug regarded as one of the most potent of the FDA approved chemotherapeutic agents. DOXO causes toxicity, especially cardiotoxicity while providing a cure in select cases, which forces the treatment to become dose-limiting DOXO cardiomyopathy is known to have a poor prognosis and is frequently fatal. DOXO causes toxic damage to the mitochondria of cardiomyocytes contributing to enhanced oxidative stress [2].

In recent years, a large number of synthetic copper(II) complexes of thiosemicarbazones ligands has been reported to act as pharmacological agents and as potential anticancer and cancer-inhibiting agents, and they have been found to be active both *in vitro* and *in vivo.* Thiosemicarbazone is a class of organic compounds that possesses a wide spectrum of biological activities and medical properties. Thiosemicarbazones contain a wide range of donor atoms and, therefore, can form coordination compounds with transition metal ions.

Thus, in the present study, we have compared the antiproliferative activity of the tested compounds with DOXO, using various cancer cell lines *in vitro*. Finally, we have also evaluated the toxicity of the tested compounds on *Daphnia magna in vivo*.

Experimental

Characterization of the tested compounds

The 2-formylpyridine *N*(4)-phenylthiosemicarbazone (*N*-phenyl-2-(pyridin-2-ylmethylidene)hydrazine-carbothioamide, complex copper(II) [Cu(L)Cl] with CMT-22 ligand (chloro(*N*-phenyl-*N*'-[(pyridin-2-yl) methylidene] carbamohydrazonothioato copper, CMT-67), mixed-ligand copper (II) complex [Cu(Str)(L)Cl] with CMT-22 and 4-aminobenzenesulfonamide (Str) ligands (chloro(N-phenyl-N'-[(pyridin-2-yl) methylidene] carbamohydrazonothioato) (4-aminobenzene-1-sulfonamide)copper, CMT-68) were synthesized in the Research Laboratory of Advanced Materials in Biopharmaceutics and Technics of the Moldova State University.

The thiosemicarbazone CMT-22 and copper(II) complex CMT-67, were synthesized as described in the literature [3]. The thiosemicarbazone CMT-22 was characterized by NMR (^{1}H and ^{13}C) spectroscopy. The complex CMT-67 was characterized by electronic, FT-IR and EPR spectroscopy, molar conductivity, magnetic susceptibility measurements and elemental analysis. Also, the crystal structure of CMT-67 was determined by single-crystal X-ray diffraction analysis. Melting points, IR, and NMR spectra of the tested compounds correspond to the literature data [3]. The copper(II) mixed-ligand complex CMT-68 was synthesized by reaction between 2-formylpyridinene (4)-phenylthiosemicarbazone (CMT-22) with $CuCl_2 \cdot 2H_2O$ and 4-aminobenze-nesulfonamide (Str) [4].

Antiproliferative activity of the tested compounds was compared with the anticancer compounds such as doxorubicin [2].

Lipinski's rule and bioactivity score

The physicochemical parameters including octanol partition coefficients (log*P*), hydrogen bond donors (nHBD), hydrogen bond acceptors (nHBA), molecular weight (Mw) and bioactivity score were calculated using molinspiration server (http://www.molinspiration.com/cgi-bin/properties) [13].

In vitro antiproliferative activity

Antiproliferative activity of the tested compounds against human melanoma cells of line MeW-164 was investigated in the Medical Research Center of the Polish Academy of Sciences using the MTT (3-(4,5-di-methylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay [5]. The line MeW-164 was derived from melanoma cell line collection established in culture from melanoma metastases, surgically removed from patients at the Warsaw Cancer Center. Investigation of the antiproliferative activity of the synthesized compounds in relation to HeLa (human cervix adenocarcinoma, ATCC CCL-2), was investigated by resazurin assay.

DNA fragmentation *in vitro*

The electrophoretic DNA fragmentation method on agarose gel, containing ethidium bromide, was used to identify the mechanism of action of the tested compounds associated with a direct effect on the genomic DNA of the cell. It was carried out by the methodology of Kumar et al. [6].

Antilipoxygenase activities assays

The LOX assay [7] system is widely employed to determine the antioxidant activity of the tested compounds. In the LOX assay, LOX-derived lipid hydroperoxides oxidize the ferrous ion (Fe^{2+}) to the ferric ion (Fe^{3+}), the latter of which binds with thiocyanate $[SCN]^-$ to generate a red ferrothiocyanate (FTC) complex. The LOX method was carried with some modifications.

In vivo acute toxicity assay

The general toxicity of the new tested compounds was evaluated using *Subphylum: Crustacea Order: Cladocera. Species: Daphnia magna.* The *Daphnia magna* originated from a culture maintained parthenoge-netically at the Institute of Zoology, Centre of Research of Biological Invasions, Laboratory of Systematics and Molecular Phylogeny [8]. The test design was based on ISO 6341: 2012. This International Standard specifies a procedure for the determination of the acute toxicity of chemical substances to the water flea *Daphnia magna*

(Straus, 1820). This method is applicable to chemical substances, which are soluble under the conditions of the test or can be maintained as a stable suspension or dispersion under the conditions of the test. The test specified in this International Standard involves the determination of the immobilization of the *Daphnia magna* after 24 h and 48 h exposure to the test sample under the conditions specified in this International Standard. The *Daphnia magna* acute mobility inhibition assay was performed using juvenile individuals of *Daphnia magna* aged up to 24 h, originating from ephippia.

The test-organisms *Daphnia magna* (Straus, 1820) (Figure 1. A) were fed with *Chlorella vulgaris* (Beijerinck, 1890) (Figure 1.B). These unicellular algae were grown using aseptic technology to exclude contamination of the culture by bacteria, algae or protozoa. The *Chlorella vulgaris* were cultivated in Prat's growth medium containing KNO_3 (1 Mm), $MgSO_4 \cdot 7H_2O$ (40 μM), $K_2HPO_4 \cdot 3H_2O$ (400 μM), $FeCl_3 \cdot 6H_2O$ (3.6 μM) in H_2O distilled (adjusted the pH to 7.0, autoclaved and stored at 5°C).

The Daphnia magna were maintained in aerated aqueous straw infusion growth media supplemented with CaCl2 (11.76 g/l), NaHCO3 (2.59 g/L), KCl (0.23 g/L), MgSO4.7H2O (4.93 g/L), (pH~7.5±0.2; O2 ≥6.0 mg/L).

Juveniles were selected according to their size and kept in fresh medium for 24 h. The Daphnia magna were cultured in Costar® 24-well culture clear sterile multiple well plates covered by a lid to prevent the possibility of contamination and evaporation but at the same time to allow gaseous exchange between air and culture medium. Each well contained 10 daphnids in 1000 μL final volume of each dilution of the tested compounds.

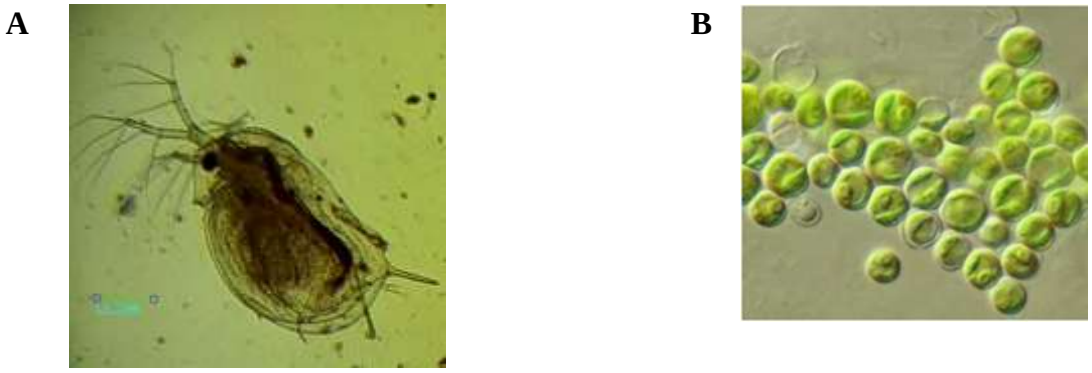


Fig.1. (A) *Daphnia magna* (Straus, 1820); **(B)** *Chlorella vulgaris* (Beijerinck, 1890).

The bioassay was then repeated at the concentrations ranging from 0.1 to 100 Mm (0.1, 1, 10, and 100 μM) in order to determine LC_{50} for each compound, including the positive control. The final test solutions contained up to 0.1% DMSO and had a final volume of 1 mL. A 0.1% solution of DMSO in aerated medium (pH~7.5±0.2; O_2 ≥6.0 mg/L) was used as a negative control and compounds were used as positive controls. Throughout the experiment, the juvenile daphnids were incubated at 22±2^{0}C, using a 16h/8h light/dark cycle (500–1000 lx). The mobility (viability) of the test organisms was observed after the 24h and 48h exposure. The experiment was performed in triplicate.

The daphnids were considered immobilized only if they did not swim during the 15s which follow gentle agitation of the test and control solutions, even if they could still move their antennae. The percentage of viability (V (%)) of *Daphnia magna* was calculated according to the formula:

$$\mathbf{V(\%)} = \frac{\mathbf{N_{(sample)}}}{\mathbf{N_{(control)}}} >$$, where N - Number of viability of *Daphnia magna.*

Results and discussions

Lipinski's rule of five is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely active drug in humans [9].

Lipinski's rule states that, in general, an active drug has no more than one violation of the following criteria [10]: no more than 5 hydrogen bond donors (nHBD), no more than 10 hydrogen bond acceptors (nHBA), a molecular weight (Mw) less than 500 daltons, an octanol-water partition coefficient (log*P*) [11] that does not

exceed 5. Log*P* as a measure of the relationship between lipophilicity and hydrophilicity of a substance. Lipophilicity affects drug absorption, bioavailability, lipophilicity drug-receptor interactions, metabolism of molecules, as well as their toxicity. Molecular polar surface area (PSA) is a very useful parameter for prediction of drug transport properties. Polar surface area is defined as a sum of surfaces of polar atoms (usually oxygens, nitrogens and attached hydrogens) in a molecule. This parameter has been shown to correlate very well with the human intestinal absorption, and blood-brain barrier penetration. Another very helpful parameter for the prediction of absorption is the polar surface area (PSA), defined as the sum of surfaces of polar atoms in a molecule, which provides good correlation with experimental transport data (intestinal absorption, Caco-2 monolayers penetration, and blood-brain barrier crossing) [11].

Table 1 lists such physicochemical properties for the tested compounds, which showed 1 violation of Lipinski rules due to a calculated the molecular weight above 500 (Table 1).

Table 1

Calculated physicochemical properties of the tested compounds

Lipinski descriptors						
COD	**log*P***	**Mw**	**nHBA**	**nHBD**	**nviolations**	**PSA**
CMT-22	2.15	256.33	4	2	0	49.31
CMT-67	-5.66	354.32	4	1	0	31.28
CMT-68	-5.65	526.53	8	5	1	108.05

On the basis of the above results, we can say that the synthesized compounds adhere to Lipinski's rule of five [12] and the exceptions to the Lipinski's rule are recognized and involve anticancer drugs such as Doxorubicin [10]. The physico-chemical properties of these new scaffolds suggest that the synthesized compounds are reasonable starting points for a drug discovery effort.

Drugs may be defined as a complex balance of various molecular properties and structure features, which determine whether particular molecule is similar to the known drugs. These properties, mainly hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility and of course presence of various pharmacophoric features influence the behaviour of molecule in a living organism, including bio-availability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others.

The bioactivity scores of the synthesized compounds were also calculated for six criteria, GPCR ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor and nuclear receptor ligand activity.

For organic molecules if the bioactivity score is more than 0.00 then the compound is active but if it is between -0.50 to 0.00 then the compound is moderately active and if the compound has less than -0.50 then it is inactive compound [13]. As we can see in Table 2, the synthesized compounds show good bioactivity score.

Table 2

Bioactivity score of the tested compounds

COD	**GPCR ligand**	**Ion channel modulator**	**Kinase inhibitor**	**Nuclear receptor ligand**	**Protease inhibitor**	**Enzyme inhibitor**
CMT-22	-0.92	-0.79	-0.94	-1.26	-0.96	-0.43
CMT-67	-0.03	0.22	-0.37	-1.09	-0.49	0.17
CMT-68	-0.05	-0.06	-0.32	-0.94	-0.08	0.24

Galaxy Visualizer allows to visualize molecular lipophilicity potential (MLP) on the molecular surface to see which parts of the surface are hydrophobic (encoded by violet and blue colors) and which hydrophilic (orange and red). MLP is calculated from atomic hydrophobicity contributions, the same that are used to calculate the octanol-water partition coefficient (log*P*). MLP is useful property to rationalize various molecular ADME characteristics (like membrane penetration or plasma-protein binding). Analysis of 3-dimensional distribution of hydrophobicity on molecular surface is particularly helpful when explaining differences in observed ADME properties of molecules with the same log*P*, since, of course, 3D parameter contains much more information then log*P* expressed by just a single value. Figure 2 displays the molecular lipophilicity potential (MLP) of the tested compounds CMT-22, CMT-67 and CMT-68.

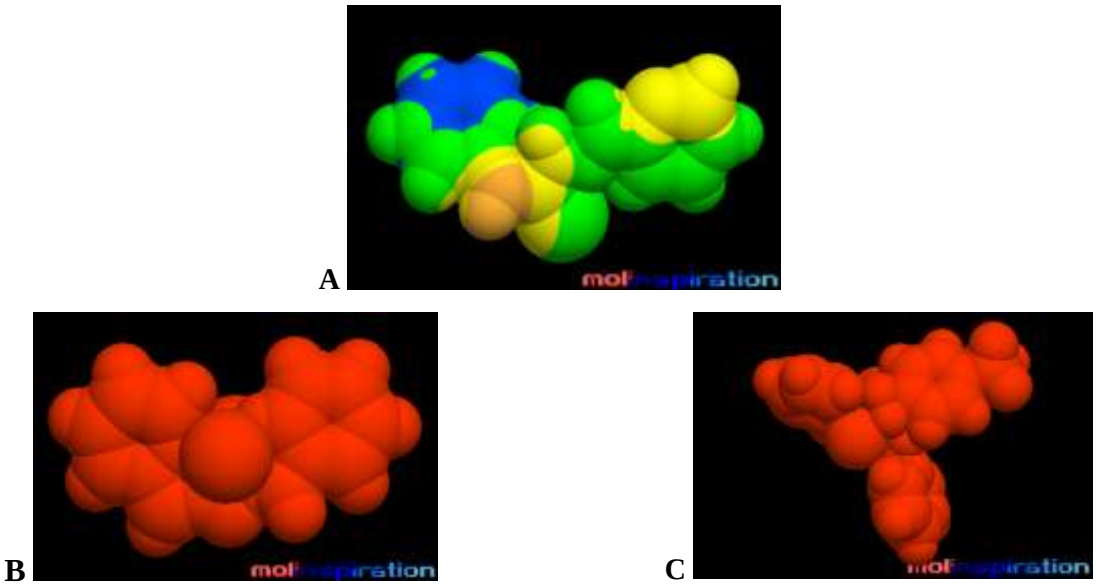


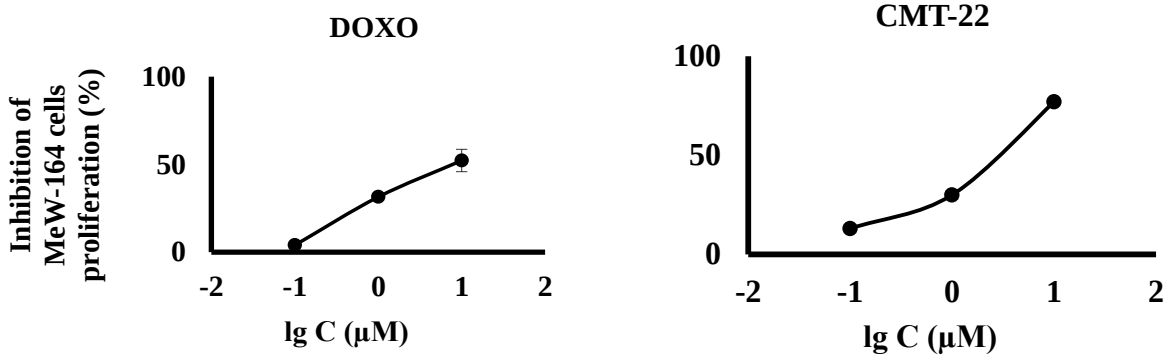
Fig.2. Molecular lipophilicity potential (MLP), virtual Log*P*, for (A) CMT-22, (B) CMT-67 and (C) CMT-68. Molecules are represented by MLP as solid colours are red/orange/yellow for hydrophilic regions; blue/violet for lipophilic regions; green for intermediate regions.

The antiproliferative activity of the tested compounds CMT-22, CMT-67 and CMT-68 on MeW-164 cells was tested, using the MTT assay [5]. The antiproliferative activity experiments was displayed in a dose-dependent manner and showed concentration dependence between the inhibitory effects of the tested compounds at the micromolar concentration range.

The tested compounds CMT-22, CMT-67, CMT-68 and the referent control DOXO possess antiproliferative activity on MeW-164 line with IC_{50} values of 2.5±0.1, 1.0±0.1, 1.0±0.1 and 7.3±0.3 μM, respectively (Fig.3). Thus, all tested compounds showed high antiproliferative activity against line MeW-164.

The viability of cells HeLa were determined by the resazurin assay [5]. The comparative study and concentration ranges identification of cytotoxic activity of CMT-22, CMT-67, CMT-68, DOXO and CDDP in regard to HeLa cells are shown in figure 4.

It was founded, that the tested compounds CMT-22, CMT-67 and CMT-68 exhibited inhibitory activity against HeLa cells, with IC_{50} values of 8.3±0.2, 2.1±0.4, and 0.40±0.04 μM, respectively. The IC_{50} values of the reference drug DOXO was found to be 10.0±0.4 μM. Thus, it was established that the copper complexes (CMT-67 and CMT-68) exhibit stronger inhibitory activity on HeLa cells proliferation than DOXO. The inhibitory activity of CMT-22 is comparable to that of DOXO.



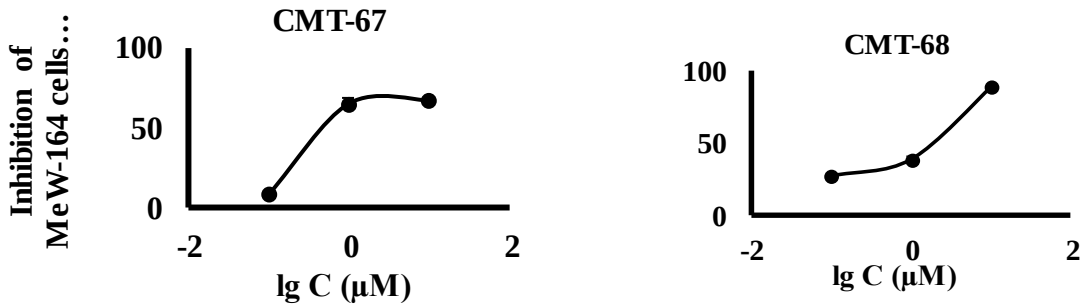


Fig.3. Antiproliferative activity of DOXO, CMT-22, CMT-67 and CMT-68 at concentrations 0.1, 1, 10 μM on MeW -164 cells. Values are represented as mean ± SD of 3 replicates.

The inhibitory rates of the copper complexes (CMT-67 and CMT-68) on cancer cells MeW-164 and HeLa proliferation manifest higher than the corresponding values of tested thiosemicarbazone CMT-22 [14]. Possibly, a much higher antiproliferative activity of copper coordination compounds can be caused by coordination of CMT-22 with the copper central atom, which leads to a change of electron density in the thiosemicarbazone moiety. So, the copper atom in these coordination compounds is able to coordinate DNA molecules.

Thus, coordination to the copper(II) ion leads to inhibition of the growth and division of cancer cells at a concentration range of 0.4-2.1 μM. It is known from the literature, that the introduction of amines into the inner sphere of copper(II) complexes with various azomethines results in an increase in their anticancer activity, and copper(II) mixed-ligand complex CMT-68 behaves as described above.

Currently, the used anticancer drugs have been shown to induce apoptosis in the cancer cells. Apoptosis is an important process of many pathological conditions. Various biochemical changes such as loss of cell membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation take place during apoptosis. The tested compounds induced apoptosis of cells were examined by DNA fragmentation of agarose gel electrophoresis and flow cytometric analysis [15].

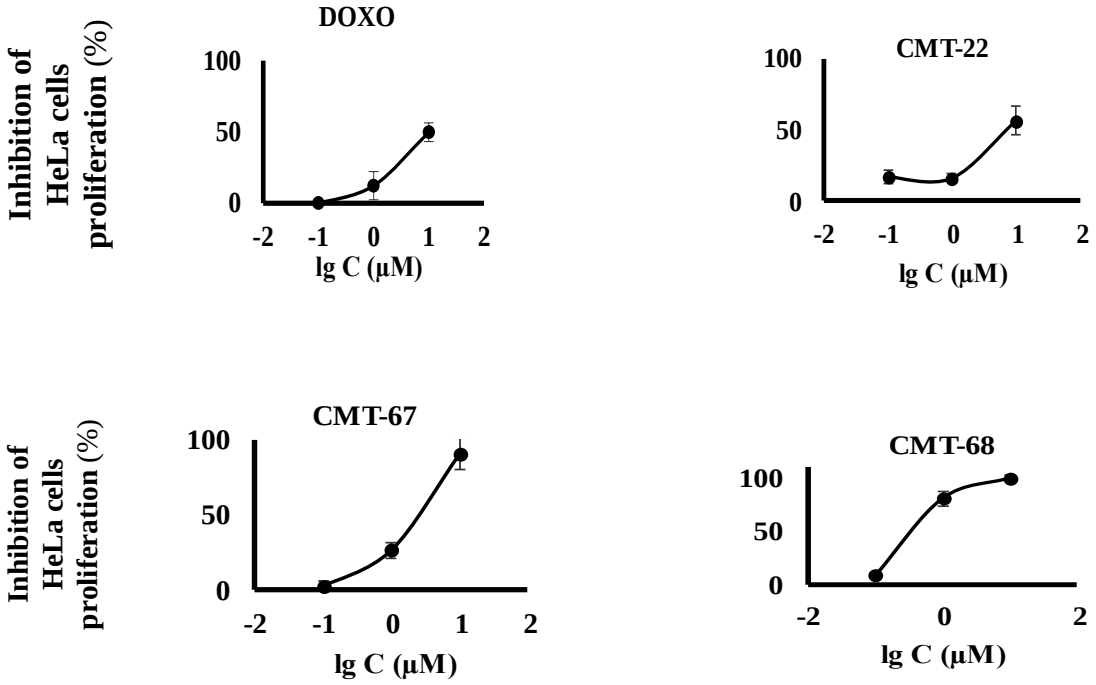


Fig.4. Antiproliferative activity of DOXO, CMT-22, CMT-67, CMT-68 at 0.1, 1 and 10 μM on HeLa cells. Values are represented as mean ± SD of 3 replicates.

Human cancer cells of line HEp-2 (ATCC CCL-23) were treated with CMT-22, CMT-67, CMT-68, CMJ-23 for 24 h. Cells of this line contain HeLa marker chromosomes, and were derived via HeLa contamination. In order to study the influence of the tested substances on DNA fragmentation, a concentration of 5 μM was used, which is average IC_{50} (μM) of all tested compounds against HEp-2 cells.

The tested compounds have demonstrated promising ability towards cleavage of genomic DNA. Thus, they have shown enhanced antiproliferative activity associated with increased induction of apoptosis by breaking the structures of the genomic DNA in the cell nucleus. It is very important, because cellular death is the underlying pharmacological purpose for chemotherapy. Disruption of the apoptotic pathways is the hallmark of cancer, being a major obstacle in chemotherapy.

Probably, the ability to induce DNA fragmentation of the mixed-ligand complex CMT-68 has resulted from its inner ligand environment properties, so the presence of an additional amino group 4-aminobenzene-sulfonamide in the internal sphere of the copper(II) mixed-ligand complex, characterized by the presence of a lone electron pair on the nitrogen atom, makes it easier to interact with the atoms of the DNA molecules grooves during replication or transcription. Possibly, a much higher antiproliferative activity of copper coordination compounds can be caused by coordination of CMT-22 with the copper central atom, which leads to a change of electron density in the thiosemicarbazone moiety. Therefore, the copper atom in these coordination compounds is able to coordinate DNA molecules.

Lipoxygenases (LOX) enzymes are reported to convert the arachidonic, linoleic, and other polyunsaturated fatty acid into biologically active metabolites that are involved in the inflammatory and immune responses. LOX also play a significant role in cancer cell growth, metastasis, invasiveness, cell survival and induction of cancer necrosis factor alpha (TNF-α) (Arfan et al., 2010). In these aspects, the ability of the tested compounds as well as DOXO to inhibit the activity of lipoxygenases was evaluated. The antioxidant quercetin was used as positive control. The tested compounds CMT-22, CMT-67, CMT-68 were able to induce inhibition of soybean LOX in a dose-dependent manner with the IC_{50} of 0.20±0.02, 0.40±0.05, 0.30±0.06 μM, respectively. In contrast, the IC_{50} for quercetin and DOXO reached values of 15.6±1.6 and 5.6±0.3 μM, respectively. Thus, these data demonstrate that the tested compounds are potent inhibitors of LOX activity (Tab.5).

Analysing the antioxidant properties of the tested compounds by LOX assays, we can see that they are potent reductive inhibitors and showed good results, comparing to DOXO and quercetin. The tested compounds showed a strong potential to be developed as new anti-inflammatory drugs.

Toxicity studies are an important stage in the development of drugs, being a prerequisite before starting their use in preclinical and clinical trials. Since the fundamental principle of toxicity studies is the protection of animals, including those participating in studies, it is currently recommended that in all possible cases, studies should be conducted on *in vivo* invertebrate organisms, avoiding the inclusion of laboratory animals in studies. *Daphnia magna* is a cladoceran organism used frequently along with *Artemia salina* (Linnaeus, 1758) in the cytotoxicity and biological activity evaluation of synthesis compounds [8].

Table 5

The percentage of inhibition of the tested compounds and positive controls on the inhibition of the lipoxygenase activity

Compound	% inhibition mean ± SD			IC_{50}
	0.05 μM	0.5 μM	5 μM	
Quercetin	20±1.5	28±2.1	50±0.8	15.6±1.6
DOXO	34.5±0.5	37.0±1.0	40.0±1.0	5.6±0.3
CMT-22	48.5±0.5	52.5±2.5	61.0±1.0	0.20±0.02
CMT-67	44.2±1.5	53.1±0.5	77.6±1.9	0.40±0.05
CMT-68	48.4±0.5	53.1±0.4	58.1±0.9	0.30±0.06

The toxicity of the tested compounds was evaluated using the *Daphnia magna* bioassay in the Institute of Zoology, Centre of Research of Biological Invasions by acad. I. Toderas et al.

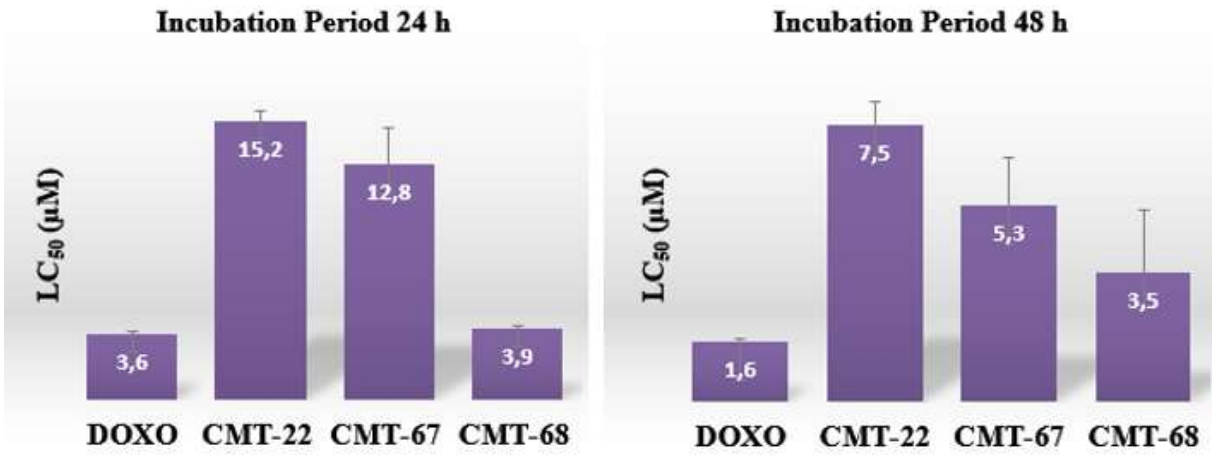


Fig.5. Toxicity on *Daphnia magna* of the tested compounds.

The results of *Daphnia magna* bioassay are given in figure 5. The tested compounds have manifested the general toxicity against *Daphnia magna* after 24h and 48h of exposure, according to the sequence: DOXO ≥ CMT-68 ≥ CMT-67 ≥ CMT-22 and DOXO ≥ CMT-68 ≥ CMT-67 ≥ CMT-22, respectively. The tested copper complexes showed promising antiproliferative activity and low toxicity on *Daphnia magna.*

Conclusions

Lipinski's rule of five, which can reasonably determine the oral bioavailability of drugs, was fulfilled by the tested compounds. All of compounds presented herein are fully compatible with Lipinski's rules and possess good TPSA, providing supporting evidence for further *in vivo* studies.

The tested compounds were also calculated for six criteria, GPCR ligand activity, ion channel modulation, kinase inhibition activity, protease inhibitor, enzyme inhibitor and nuclear receptor ligand activity, and have shown good bioactivity score. However, these techniques have limitations and not all molecules can be benefited.

The inhibitors of cancer cell proliferation (CMT-22, CMT-67, CMT-68) characterized by high selective activity, low toxicity and higher efficiency compared to DOXO have been identified, which opens up prospect of their employment as anticancer agents. The tested compounds have been found to manifest a high antiproliferative activity towards cancer cells MeW-164, HeLa that in most cases is by 1.2-100 times higher than that of DOXO. It was revealed that the mechanism of action of antiproliferative activity of the tested compounds is associated with apoptosis of cells.

The ability of the tested compounds to inhibit the LOX activity in comparison with quercetin and DOXO is more essential according to the rank order CMT-22 ≥ CMT-67 = CMT-68 ≥ DOXO ≥ quercetin.

The tested compounds are less toxic than DOXO for *Daphnia magna*. Thus, the tested compounds showed promising antiproliferative activity against cancer cells and low toxicity on *Daphnia magna.*

References:

1. BRAY, F., JEMAL, A., GREY, N., FERLAY, J., FORMAN, D. Global cancer transitions according to the Human Development Index (2008-2030): a population-based study. In: *Lancet Oncol.*, 2012. vol.13, no.8, pp.790-801.
2. TACAR, O., SRIAMORNSAK, P., DASS, CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. In: *Pharm. Pharmacol*., 2013, vol.65, no.2, pp.157-70.
3. GRAUR, V., GARBUZ, O., ZARICIUC, E., GUDUMAC, V., GULEA, A. Synthesis and biological activity of 2-formylpyridine N(4)-allyl-3-selenosemicarbazone and its copper coordination compound. In: *Journal of Academy of Sciences of Moldova. Life Sciences*, 2015, vol.3, no.327, pp. 135-142.
4. ILIESAB, D., SHOVAC, S., RADULESCUB, V., PAHONTUD, E., ROSUA, T. Synthesis, characterization, crystal structure and antioxidant activity of Ni(II) and Cu(II) complexes with 2-formilpyridine N(4)-phenylthiosemicarbazone. In: *Polyhedron*, 2015, vol.97, pp.157-166.
5. PAHONTU, E., FALA, V., GULEA, A., et al. Synthesis and Characterization of Some New Cu(II), Ni(II) and Zn(II) Complexes with Salicylidene Thiosemicarbazones: Antibacterial, Antifungal and in Vitro Antileukemia Activity. In: *Molecules*, 2013, vol.18, pp.8812-8836.

6. LIM, SW., LOH, HS., TING, KN., et al. Reduction of MTT to Purple Formazan by Vitamin E Isomers in the Absence of Cells. In: *Trop. Life Sci. Res*., 2015, vol.26, no.1, pp.111-20.
7. KUMAR, K., DEKA, D., SINGH, A., MITRA, D.K., VANITHA, B.R., AND DADA, R. Predictive value of DNA integrity analysis in idiopathic recurrent pregnancy loss following spontaneous conception. In: *Assist. Reprod. Genet.*, 2012, no.29, pp.861-867.
8. KHAN, I., NISAR, M., EBAD, F., NADEEM, S., SAEED, M., et al. Anti-inflammatory activities of Sieboldogenin from Smilax china Linn.: experimental and computational studies. In: *Ethnopharmacol*, 2009, no.121, pp.175-7.
9. GARBUZ, O., TODERASH, I., ULCHINA, I., GRAUR, VO., RAILYAN, N., GULEA, A. The Antiproliferative, Antioxidant Activities and Toxicity of Mixed-Ligand Amine-Containing Copper (II) Coordination Compounds with 2-(2-Hydroxybenzylidene)-N-(Prop-2-En-1-Yl) Hydrazinecarbothioamide. In Conference: *"Sustainable use and protection of animal world in the context of climate change"*. Chișinău, Moldova, 16-17 septembrie 2021 pp.123-129.
10. LIPINSKI, C.A., LOMBARDO, F., DOMINY, B.W., FEENEY, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. In: *Advanced Drug Delivery Reviews*, 1997, no.23, pp.4-25.
11. LIPINSKI, C.A., LOMBARDO, F., DOMINY, B.W., FEENEY, P.J. "Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings". In: *Advanced Drug Delivery Reviews*, 2001, no.46 (1–3), pp.3–26. doi:10.1016/S0169-409X(00)00129-0.PMID11259830.
12. LEO, A., HANSCH, C., ELKINS, D. Partition coefficients and their uses. In*: Chem Rev*. (1971) no.71 (6), pp.525–616. doi:10.1021/cr60274a001.
13. WANI, M.Y., BHAT, A.R., AZAM, A., ATHAR, F. EUR. In: *Med. Chem*., (2013) no.64, pp.190-199.
14. SHAMSUZZAMAN, KHAN A.A. ABDUL BAQI, ABAD ALI, MOHD ASIF, ASHRAF MASHRAI, HENA KHANAM, ASIF SHERWANI, ZAHID YASEEN, MOHAMMAD OWAIS. Synthesis, characterization, biological evaluation and molecular docking of steroidal spirothiazolidinones. In: *Journal of Molecular Structure*, 2014, pp.1-29 http://dx.doi.org/10.1016/j.molstruc.
15. GULEA, A., GUDUMAC, V., GARBUZ, O., ANDRONACHE, L. In vitro antiproliferative activity and antioxidant capacity of new organometallic coordination compounds, results correlation analysis. În: *Buletinul AȘM*. Seria „*Științele vieții*", 2016, vol.3, nr.330, p.65-72.
16. ГУЛЯ, А., ГРАУР, В., ЧУМАКОВ, Ю., ПЕТРЕНКО, П., ГАРБУЗ, О., ЦАПКОВ, В., ГУДУМАК, В. Координационные соединения никеля(II) с бис(4-аллилтиосемикарбазоном) пентан-2,4-диона. В: *Журнал общей химии*, 2020, том 90, №1, с.143-150.

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Prezentat la 15.10.2021