Conference paper

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Novel selective anticancer agents based on Sn and Au complexes. Mini-review

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Abstract: Cancer is one of the most common causes of death in modern medicine. Molecular design of novel substances with pharmacological activity is one of the goals of medicinal inorganic chemistry. Platinum complexes are widely used in the treatment of cancer, despite high efficacy their use is limited by side effects, as well as primary or acquired resistance. In this regard, the search for novel metal-containing antitumor compounds is underway. Organotins and gold compounds are promising pharmacological agents with anti-cancer properties. The introduction of protective antioxidant fragments into inorganic compounds molecules is a way to reduce the side effects of anti-cancer drugs on healthy cells. 2,6-dialkylphenols belonging to vitamin E (α-tocopherol) mimetics are widely used as antioxidants and stabilizers. The properties of Ph_3SnCl (**Sn-I**), Ph_3PAuCl (**Au-I**) and complexes Ph_3SnSR (**Sn-II**) and Ph_3PAuSR (**Au-II**) based on 2,6-di-*tert*-butyl-4-mercaptophenol (**RSH**) as radical scavengers and reducing agents were studied in model reactions. For **Sn-II** and **Au-II** the comparative study of cytotoxic action was made and the IC_{50} values on different cancer cell lines were found to be depended on the nature of metal. In general, Sn(IV) complexes possessed higher cytotoxicity than Au(I) complexes. In order to clarify the mechanism of cytotoxic mode of action the effect of compounds on Fe^{3+}-induced lipid peroxidation, mitochondrial potential and mitochondrial permeability, cell cycle and induction of apoptosis was studied. Organotin compounds can bind tubulin SH-groups and inhibit its polymerization by a dose-dependent mechanism, whereas gold compounds inhibit *Thioredoxin reductase* (*TrxR*). *In vivo* experiments on acute toxicity of **Sn-II** and **Au-II** proved their moderate toxic action that opens prospects for the further study as antitumor agents.

Keywords: 2,6-di-*tert*-butylphenol; antioxidants; apoptosis; Au(I) complexes; cell cycle; cytotoxicity; Mendeleev-21; metal-based drugs; organotin compounds; toxicity *in vivo*.

Introduction

The discovery of the anticancer properties of cisplatin and its analogues (oxaliplatin and carboplatin) – Pt (II) compounds, their wide clinical success in modern cancer treatment methods have caused great interest in the study of anticancer drugs based on metal compounds [1].

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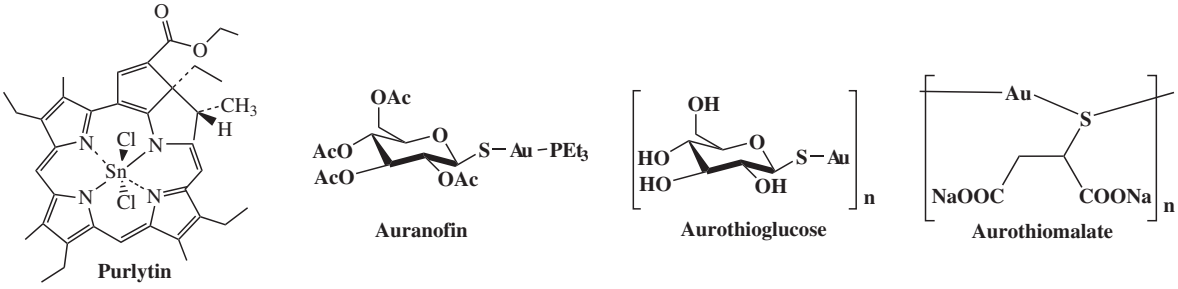
Compounds of the general formula R_nSnX_{4-n}, (X = Cl, Br) contain a Sn(IV) atom and are classified as mono-, di-, tri-, and tetraorganotins depending on the number of alkyl or aryl groups. The rapid growth of industrial, agricultural, and biological uses of organotin compounds over the past few decades has led to an increase in their concentration in the environment and in biological systems [2]. Since organotins molecules contain lipophilic organic groups, they are able to easily penetrate through the cell membrane, which leads to a rapid increase in their concentration in lipids. Migrating along the trophic chain, these toxicants pose a threat to human health [3]. In addition, Sn is a biogenic element necessary for calcification processes; therefore, the correct development of the skeleton is impossible without it. The biological activity of Sn(IV) compounds is sharply different from the action of elemental tin and Sn(II) inorganic salts. An intensive study of organotin compounds began after the discovery of their antitumor activity by M. Gielen in the 1980s. The researcher's attention was attracted mainly by organotin compounds which possess high antitumor activity that opens up the possibility of their study as promising pharmacological substances [4–8]. Organotin(IV) carboxylates with o- or p-hydroxybenzoic acids showed high cytotoxicity against sarcoma cancer cells [9]. The Bu_3Sn and Ph_3Sn-complexes with 2-thiobarbituric acid demonstrate higher cytotoxic activity than that of cisplatin against human breast adenocarcinoma cells (MCF-7), their IC_{50} values were 272 and 179-fold lower than that of cisplatin, respectively [10, 11].

It is well known that the Sn atom interacts with free SH groups in proteins, which leads to distortion of the structure and loss of protein functionality. Organotin chlorides can promote lipid peroxidation in cell membranes and cause oxidative stress [12]. It has been shown that R_nSnX_{4-n} exhibiting antitumor activity bind and unwind DNA, affect cell death as a result of necrosis, increase the influx of extracellular Ca^{2+} and generate reactive oxygen species (ROS) that leads to apoptosis [13]. Compounds Sn(IV) act on cells by various mechanisms: changes in membrane permeability, suppression of the oxidative phosphorylation and ATP synthesis in mitochondria, accumulation in the Golgi apparatus and endoplasmic reticulum, influence on DNA, RNA and protein synthesis, induction of apoptosis [14]. Studies of the binding of DNA to organotin compounds are of great importance for the production of new antitumor agents [15]. Moreover, the correlation between anti-tumor action of organotin(IV) complexes with thioamide ligands and inhibition of enzyme *lipoxygenase* by compounds in catalytic oxidation of linoleic acid to hydroperoxylinoleic acid was discovered [16–18]. The tin drug Purlytin (Rostaporfin) is used in clinical practice (Scheme 1) [19]. Purlytin – tin etiopurpurin – belongs to the class of metal complexes with tetrapyrrole macrocycles as ligands, and is used as a photosensitizer to treat age-related macular degeneration. The drug was approved by the FDA in 2004 for therapy in ophthalmology. However, *Miravant Medical Technologies* is currently conducting phase II/III clinical trials for PDT for metastatic breast cancer.

Among several classes of metal complexes (Ru, Pd, Ti, Cu, Au, etc.) which were noted as promising anticancer agents, many studies focus on various gold compounds in oxidation states +1 and +3. This is because the compounds Au(I) are considered as possible antiproliferative agents in connection with their long-term use in the clinic for the treatment of rheumatoid arthritis. Au(III) compounds form flat square complexes, isoelectronic and isostructural Pt (II) complexes, due to which they can have a similar biological effect.

Gold and its complexes have historically been used to treat a wide range of diseases, but the rational use of Au-based compounds in medicine began in the early 1920s, when the bacteriostatic effect of the



Scheme 1: Examples of Sn(IV) and Au(I) drugs purlytin, auranofin, aurothioglucose and aurothiomalate.

metal *in vitro* was proved. The characteristic oxidation states for gold are Au(I) and Au(III), and Au(0) is found in colloidal solutions. The first known gold salt acting as a drug was gold cyanide $K[Au(CN)_2]$, used by bacteriologist Robert Koch to kill mycobacteria, the causative agent of tuberculosis [20]. In 1929, J. Forestier proposed the use of gold salts for the treatment of rheumatoid arthritis [21]. Gold compounds such as auranofin, aurothioglucose and aurothiomalate in which gold is bound to a sulfur atom are widely used as antirheumatic drugs (Scheme 1).

Water-soluble compounds are used in the clinic and contain hydrophilic groups (e.g. glucose) as well as an aurothiogroup (–S–Au–). The high affinity for sulfur and the inhibitory effect of gold salts on various enzymes suggested that the therapeutic effect of Au compounds may be due to inhibition of binding with sulfhydryl residues [22]. Recent studies have shown that auranofin and other gold compounds bearing biologically relevant molecules demonstrate antitumor properties and they are able to overcome cisplatin resistance in cancer cells of the human ovary, confirming the suggestion that the mechanism of action of this compound is different from the damage to DNA caused by cisplatin [23]. In particular, it was found that auranofin acts as a powerful inhibitor of thioredoxin reductase (TrxR) and causes a change in the cell redox status, which leads to an increase in the production of superoxide radical anion, H_2O_2 and oxidation of the components of the thioredoxin (Trx) system [24–26].

The *lipoxygenase* inhibition and binding affinity towards the calf thymus DNA by triphenylphosphine gold(I) complexes with the thioamides was studied [27–29].

Main problems in creating drugs are the rational "activity-toxicity" ratio and the reduction of side effects. The development of organometallic therapeutic agents is hampered by their toxicity. Since Sn(IV) and Au(I) in auranofin analogs can induce oxidative stress leading to cytotoxicity, many attempts have been made to minimize the pro-oxidant effect and generation of reactive oxygen species (ROS) [30]. Including of additional 2,6-di-*tert*-butylphenol group in organotin complexes decreased the cytotoxicity of compounds [31]. These results indicated that attenuation of pro-oxidative properties of tin and gold containing drugs by introduction of radical scavenging moiety is a key prerequisite for the rational design of anticancer therapeutics with balanced redox potential [32]. Hence, numerous studies are being conducted on the modification of approved preparations based on metal compounds, including gold and tin. To reduce the side effects of anticancer drugs on healthy cells the introduction of substituents with protective (antioxidant) activity in the molecule seems reasonable.

Antioxidants play an important role in the regulation of free-radical processes in the body. One of the most promising directions in the chemistry of antioxidants is the synthesis of hybrid compounds combining antioxidant activity with the ability of structural interaction with a biosystem. Such compounds include 2,4- and 2,6-dialkylsubstituted phenols, in particular, 2,6-di-*tert*-butylphenols. These compounds exhibit a wide range of biological activity: anti-inflammatory, antimicrobial, antiviral, etc. The antioxidant defense system in a living body regulates fluctuations in the prooxidant-antioxidant balance and protects cells from damage caused by high levels of oxidative stress. Analogues of the natural antioxidant α-tocopherol are widely used as inhibitors of the formation of free radicals in the oxidative destruction of natural and synthetic substrates [33]. Phenolic compounds such as 2,6-di-*tert*-butyl-4-methoxyphenol (BGT, E320) and 2,6-di-*tert*-butyl-4-methylphenol (BHT, E321) are examples of some synthetic antioxidants. Their activity is related with mobility of the hydrogen atom of the phenolic OH group, which is easily cleaved by the action of peroxyl radicals, forming a relatively unreactive phenoxyl radical, breaking off the radical chain process [34].

Sterically hindered phenols inhibit effectively the oxidation of various organic substrates [35]. In the case of sterically hindered phenols, the formation of hydrogen bonds is impossible. The effectiveness of 2,6-di-*tert*-butylphenols as inhibitors of the oxidative destruction of hydrocarbons is determined by the nature of the group in the para position of the aromatic ring, which affects the stability of the corresponding phenoxyl radicals formed during the oxidation process [36]. It is worth to note that in cancer organs and tissues where the pH value is close to five the principal OH group of phenolic antioxidants is protonated that can bock the activity of antioxidant in cancer media. On the other hand, in normal tissues and cells at physiological pH the antioxidant is active.

Our group have reported the antiproliferative activity of organotin(IV) complexes bearing 2,6-di-*tert*-butylphenol fragment with heterocyclic thioamides. The IC_{50} value for bis-(3,5-di-*tert*-butyl-4-hydroxyphenyl)

tin complex $R_2Sn(MPMT)_2$ (where MPMT = 2-mercapto-4-methylpyrimidine, R = 3,5-di-*tert*-butyl-4-hydroxyphenyl) was 32-fold more cytotoxic than cisplatin at MCF-7 cancer cells [37]. In order to lower the toxic effect of organotins the use of antioxidants is suggested. A series of organotin complexes based on 2,6-di-*tert*-butyl-4-mercaptophenol were synthesized and tested against cancer and normal cells, also the ability of complexes to decrease the content of SH groups in tubulin allows one to consider these compounds as potential antimitotic agents. Complexes exhibited significantly lower cytotoxic activity against normal MRC-5 cell line compared to the tumor cell lines MCF-7 and HeLa [31].

Living organism possesses sophisticated protective antioxidant system, which prevents oxidative stress. It consists of low-molecular natural antioxidants (ascorbic acid, tocopherols, lipoic acid, and carotenoids, etc.) and enzymatic antioxidants such as superoxide dismutase (SOD), catalase, glutathione and glutathione dependent enzymes, regulates the toxic impact of ROS and prevents cell damage. There are several mechanisms of antioxidants action depending on the interaction of antioxidative agents with various ROS: a dismutation of superoxide radical-anion by SOD, scavenging of hydroxyl radical $HO^{\cdot}$, interaction either with peroxyl radicals of lipids $LOO^{\cdot}$ or lipid hydroperoxides LOOH breaking down the chain radical process of lipid peroxidation. Recent results in testing metal complexes with 2,6-di-*tert*-butylphenol moiety showed that they could potentially facilitate the scavenging of excess ROS, and thus normalize redox balance in the damaged cells and organs [38].

In this regard, the study of the biological activity of tin and gold compounds with 2,6-di-*tert*-butylphenol fragment of urgent importance.

Biological activity of Sn(IV) and Au(I) thiolates

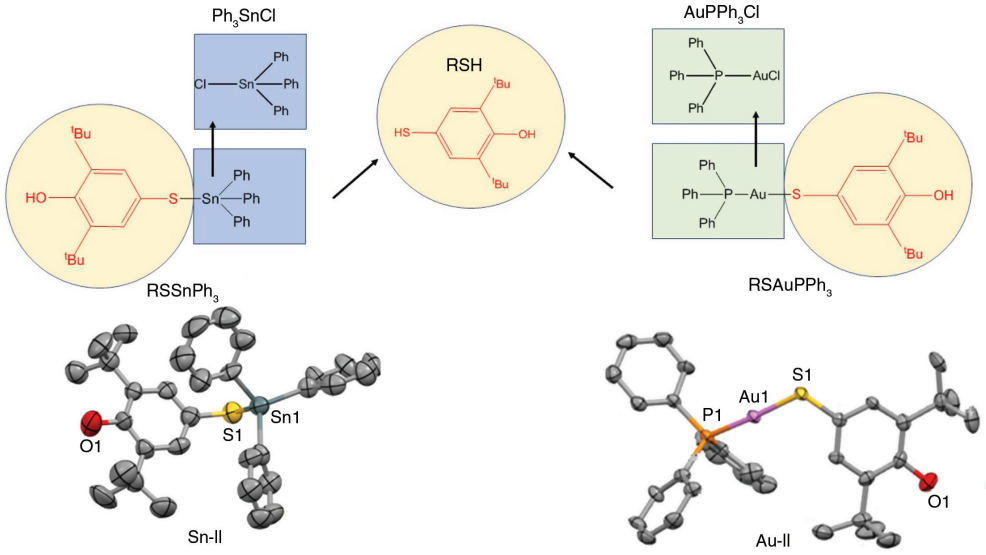
The activity of organotins is determined by: (1) the nature of the substituents; (2) the coordination availability of the Sn atom to form a bond with the target; (3) a relatively stable L–Sn bond (for example, S–Sn); (4) slow hydrolytic destruction [39]. Synthesis and complex study of the biological activity of Sn and Au complexes containing antioxidant 2,6-di-*tert*-butylphenol fragment was carried out recently. The structures of compounds (Scheme 2) were confirmed by ^1H, ^{13}C NMR, X-ray diffraction and elemental analysis. The mechanisms of their biological action and "structure – activity" correlations has been intensively studied. A comparative study of precursors **Ph_3SnCl** (**Sn-I**), **$AuPPh_3Cl$** (**Au-I**) and 2,6-di-*tert*-butylphenol (**RSH**) and their complexes **Ph_3SnSR** (**Sn-II**) and **Ph_3PAuSR** (**Au-II**) in the model processes of interaction with ROS and electron transport was carried out. Cytotoxicity *in vitro* on human cancer cell lines, cell cycle and influence of various structural fragments on the activity of compounds were studied [31, 32, 40].

Antioxidant properties in model systems

The study of redox activity using the CUPRAC test

The redox activity was studied in CUPRAC (Cupric Ion Reducing Antioxidant Capacity) test [41]. This method is based on the reduction of Cu(II) ions by the studied compound forming Cu(I) complex with neocuproin (2,9-dimethyl-1,10-phenanthroline), which has a maximum absorption at 450 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as standard, the results for complexes and starting compounds are presented in Trolox equivalents antioxidant capacity (TEAC) (Table 1) [32, 42].

The initial chlorides **Au-I** and **Sn-I** are inactive in the Cu(II) reduction. The most active reducing agent was **Sn-II** for which the Trolox equivalent was 1.7 times higher than Trolox and 1.4 times higher than organic precursor **RSH**. Introduction of a phenolic fragment in gold compound **Au-II** resulted in less antioxidant activity than the **Sn-II**. It can be assumed that $SnPh_3$ fragment increases the stability of the phenoxyl radical is responsible for the antioxidant activity.



Scheme 2: Structures of tin and gold complexes based on 2,6-di-*tert*-butyl-4-mercaptophenol. Molecular structure of compounds **Sn-II** and **Au-II** (hydrogen atoms are omitted for clarity).

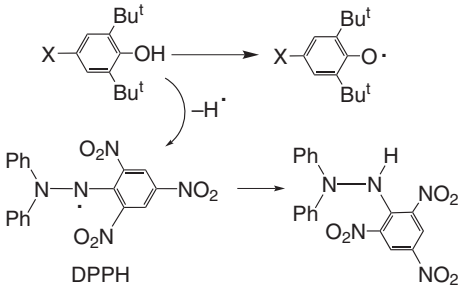
Table 1: TEAC values for the studied compounds and Trolox.

Compound	Trolox	RSH	Au-I	Au-II	Sn-I	Sn-II
TEAC	1.00 ± 0.03	1.24 ± 0.03	0.12 ± 0.02	1.05 ± 0.07	0.23 ± 0.02	1.69 ± 0.13

DPPH-test

The radical scavenging activity of compounds has been studied in the process of hydrogen atom transfer from the phenol moiety to the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to give diphenylpicrylhydrazine and phenoxyl radicals (Scheme 3) which can undergo further reactions such as coupling, fragmentation and addition [43].

Antiradical activity was evaluated as the amount of antioxidant necessary to decrease the initial concentration of DPPH by 50 % (Efficient Concentration = EC_{50}). The EC_{50} values for **Sn-II** and **Au-II** were 15 ± 4 and 31 ± 2 μM, respectively, whereas the activity of **RSH** was several times lower (55 ± 10 μM). Compounds **Au-I** and **Sn-I** were not active in this test since they do not possess active OH group. The antioxidant activity depends on metal atom nature in the phenol containing complexes. Thus, the antioxidant activity of gold and tin compounds bearing hindered phenol group is mainly determined by the ability to hydrogen atom abstraction from the –OH group and such compounds possess radical scavenging activity.



Scheme 3: Mechanism of radical scavenging activity of 2,6-di-tert-butylphenols with DPPH.

Evaluation of the ability of compounds to react with NADPH

NADPH affects the pool of adenine nucleotides which plays an important role in antioxidant defense system. The ability of compounds to oxidize NADPH was monitored spectrophotometrically in the presence of NADPH at λ_{max} 340 nm for 30 min. Since the absorbance and structure of the spectra did not change during incubation, the absence of a reaction between the substances and NADPH can be postulated.

Reaction with ROS

Interaction with superoxide radical anion $O_2^{\bullet-}$

The activity of the complexes with respect to the superoxide radical anion $O_2^{\bullet-}$ generated in the xanthine/xanthine oxidase enzymatic system was determined by the change in the reduction rate of tetrazolium blue to formazan (Fig. 1) [44]. All compounds were practically inactive, the initial **Au-I** gold complex showed little activity (Fig. 1b).

Interaction with hydroxyl radical $^{\bullet}OH$

The oxidation of Fe^{2+} ions by hydrogen peroxide (Fenton reaction) was used to generate the hydroxyl radical which reacts with *D*-deoxyribose to form malonic dialdehyde (MDA), whose concentration was determined spectrophotometrically by reaction with thiobarbituric acid leading to trimethine complex with maximum absorption λ_{max} 532 nm [45]. The results are presented in % of control (Table 2). The antioxidant ionol (BHT) was used for comparison.

Since **RSH** demonstrates high antioxidant activity, the introduction of the RS fragment into the organotin complex increases its activity, the opposite situation is observed for the **Au-II**. Apparently, for the initial **Au-I**, a ligand exchange reaction involving $^{\bullet}OH$ can take place.

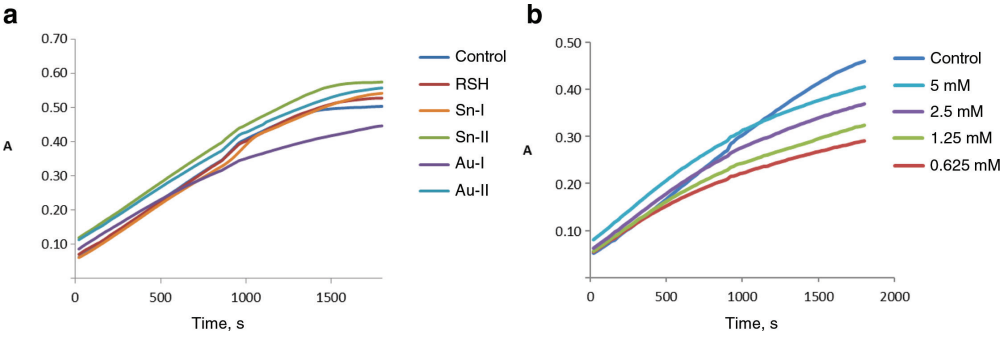


Fig. 1: Kinetic curves of formazane accumulation in the xanthine/xanthine oxidase system in the presence of different compounds (a) and different concentrations of the **Au-I** (b).

Table 2: The activity of the studied compounds and ionol in the reaction with $^{\bullet}OH$.

Compound	ionol	RSH	Au-I	Au-II	Sn-I	Sn-II
Activity, %	77	74	83	112	103	88

Assessment of the ability of compounds to react with H_2O_2

Spectrophotometric monitoring of the reactivity of the studied compounds with respect to H_2O_2 in the 200–350 nm range [46] showed no changes in the structure of the spectra during incubation (10 min) indicating the absence of interaction between substances.

Fe^{3+}-induced lipid peroxidation

Mitochondria are the main source of intracellular ROS generation. Abnormal level of ROS causes the oxidative damage of intracellular structures as proteins, nucleic acids, lipids, membranes and other biomolecules with consequent injury to cells. ROS are proposed to be involved in all the steps of carcinogenesis [38]. Lipid peroxidation of mitochondrial membranes leads to disturbances of oxidative phosphorylation, a major source of energy. The monitoring of lipid peroxidation in mitochondrial suspension can be followed by the accumulation of substances that reacted with thiobarbituric acid (TBARs) [47]. Oxidative damage (lipid peroxidation) was induced by Fe^{3+}. **RSH** was found to be effective antioxidant with (IC_{50} 0.2 μM), whereas **Sn-II** demonstrated moderate antioxidant activity (IC_{50} 3.1 μM) unlike **Sn-I** with prooxidant properties. This effect is observed most likely due to the presence of a metal atom (Sn) in their structure [48].

The influence of gold complex **Au-II** and their precursors **RSH** and **Au-I** on lipid peroxidation of isolated rat brain mitochondria was studied. In contrast to **Au-I**, **RSH** and the **Au-II** similarly decreased the TBARs concentration upon Fe^{3+}-induced lipid peroxidation of mitochondria (Fig. 2). The tendency to decrease the TBARs level in the presence of phenolcontaining complexes and **RSH** leads one to suggest the main role of the phenol moiety in the antioxidant mechanism.

Toxicity of Sn and Au compounds

MTT test

Cytotoxicity of compounds **RSH**, **$RSAuPPh_3$** (**Au-II**), **$RSSnPh_3$** (**Sn-II**), their precursors **$ClAuPPh_3$** (**Au-I**), **$ClSnPh_3$** (**Sn-I**), as well as mixtures of **RSH** with **Au-I** and **Sn-I** in 1:1 ratio were studied by MTT test on the cancer cell lines MCF-7, HCT-116 and A549 (lung cancer) and conditionally normal cells MCF10A (breast epithelial cells) and VA13 (embryonic lung fibroblasts). Cell survival curves were obtained and IC_{50} values

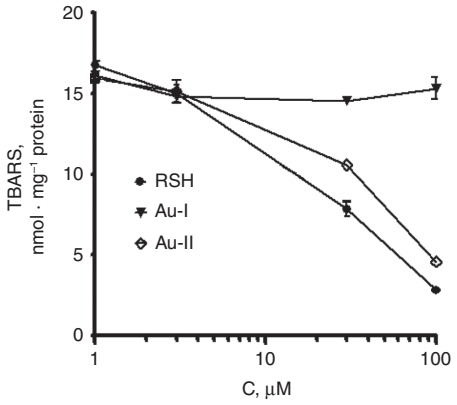


Fig. 2: Influence of gold complex **Au-II** and precursors (**RSH** and **Au-I**) on Fe^{3+}-induced lipid peroxidation of isolated rat brain mitochondria.

were determined (Table 3). The introduction of a phenolic fragment in the case of **Au-II** reduced the toxicity compared to precursors **Au-I** and **Sn-I**. **RSH** and **Au-II** demonstrated low toxicity up to >50 μM. Moreover, the study of mixtures of **Sn-I** and **RSH** showed no significant change. **Sn-II** was 6 times less toxic in comparison with **Sn-I** at the HCT-116 cells. Consequently its toxicity was studied on other cancer cell lines (Table 4) [42].

Study of apoptosis and cell cycle

The effect of compounds on cell death of cultured cells during incubation for 48 h with compounds at $2 \cdot IC_{50}$ concentration was studied. After incubation, the cells were removed from the substrate, washed and the percentage of apoptotic and necrotic cells was detected using a cytofluorimetry. This analysis is base on the detection of phosphatidylserine (PS) on the surface of apoptotic cells using fluorescently labeled Annexin V in combination with the dead cell marker 7-AAD [49]. Annexin V is a Ca^{2+}-dependent phospholipid binding protein that has a high affinity for PS, a membrane component usually localized to the inner surface of the cell membrane [50]. At the beginning of apoptosis, PS molecules move to the outer surface of the cell membrane, where Annexin V can easily bind them. At the late stage apoptotic cells show a loss of membrane integrity. Membrane-impervious dye 7-AAD is used to distinguish dead cells from early apoptotic cells. Analysis of apoptotic profile can distinguish four cell populations:

(1) Viable cells that do not undergo apoptosis: Annexin V (−) and 7-AAD (−); (2) Early apoptotic cells: Annexin V (+) and 7-AAD (−); (3) Late apoptotic cells: Annexin V (+) and 7-AAD (+); (4) Cells that have died by non-apoptotic pathway: Annexin V (−) and 7-AAD (+). The obtained results of the distribution of 7-AAD-positive and negative and AnV-positive and negative cells in % are presented in Fig. 3 and Table 5 for MCF-7 and HCT-116 cells.

For the MCF-7 cells **Au-I** and **Au-II** complexes after 48 h of incubation the percentage of late apoptotic cells is maximal. However, there are no significant differences in the percentage of cells in the pool of early and late apoptotic cells for the **Au-I** compound and **Au-II** with phenol phragment. The **RSH** does not induce tumor cell apoptosis. It was shown previously that the **Sn-II** induces not only depolarization of mitochondria

Table 3: IC_{50} values for the studied compounds on HCT-116 and MCF-7 cell lines.

Compound	IC_{50}, μM	
	HCT-116	MCF-7
RSH	>50	>50
Au-I	7.7 ± 1.3	12.1 ± 1.5
Au-II	>50	>50
Sn-I	0.01 ± 0.001	0.07 ± 0.01
Sn-II	0.06 ± 0.01 [42]	0.09 ± 0.01 [42]
Cisplatin	6.5 ± 0.5	18.5 ± 0.2 [31]

Table 4: The IC_{50} values for the compounds of **Sn-II** and cisplatin on different cancer cell lines [42].

Compound	IC_{50}, μM				
	CaCo-2	SNB-19	A-498	M-14	NCl-H332M
Sn-II	0.09 ± 0.02	0.06 ± 0.03	0.04 ± 0.01	0.15 ± 0.03	0.10 ± 0.01
Cisplatin	62.3 ± 1.2	0.81 ± 0.03	17.5 ± 1.2	10.8 ± 4.5	2.7 ± 0.3

Colon (CaCo-2), glioblastoma (SNB-19), renal (A498), melanoma (M14), non-small cell lung (NCI-H322M) cell lines.

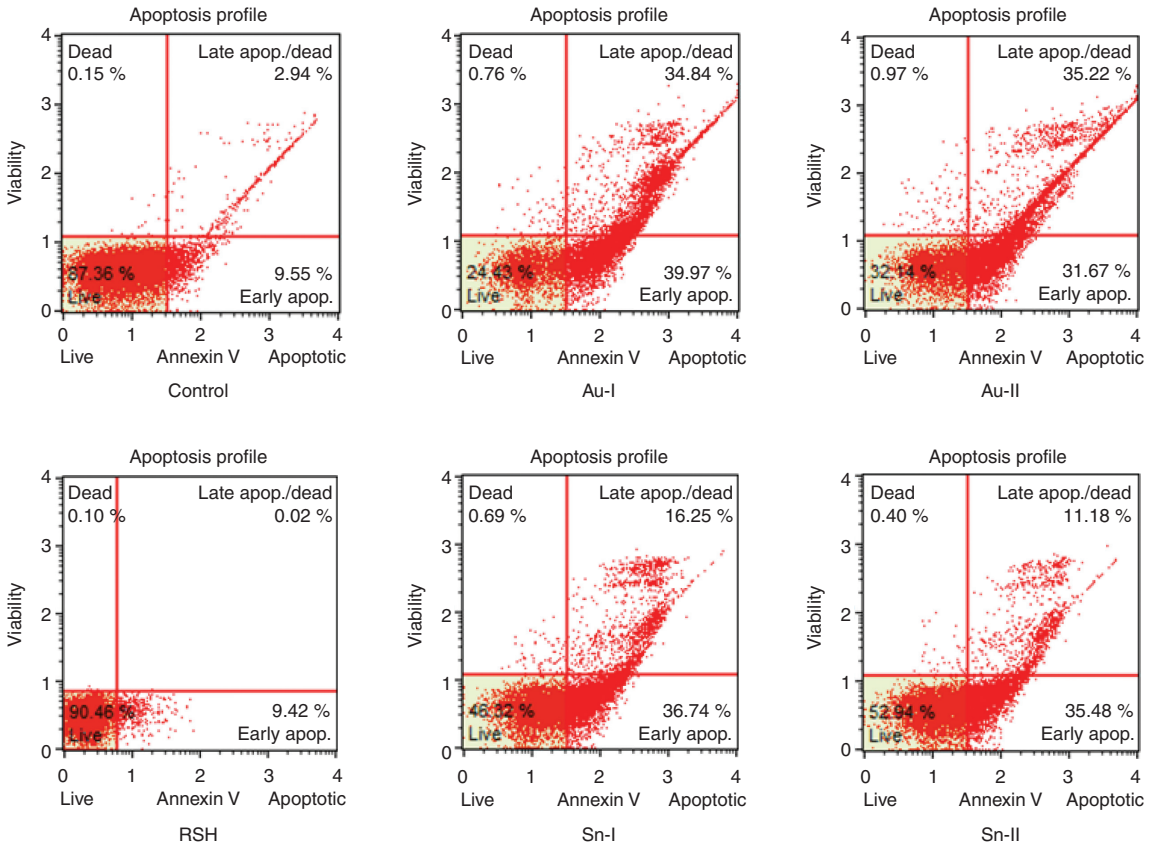


Fig. 3: Apoptotic profile of cancer cells MCF-7 after treatment with different compounds at μM after 48 h. Concentration of compounds $2 \cdot IC_{50}$.

Table 5: Distribution (%) of 7-AAD-positive and negative and Annexin V (AnV)-positive and negative MCF-7 and HCT-116 cells.

Compound	AnV(–)/7-AAD(–)	AnV(+)/7-AAD(–)	AnV(–)/7-AAD(+)	AnV(+)/7-AAD(+)
MCF-7				
Control	87.4 %	9.5 %	0.2 %	2.9 %
RSH	90.5 %	9.4 %	0.1 %	0.1 %
Au-I	24.4 %	40.0 %	0.8 %	34.8 %
Au-II	32.1 %	31.7 %	1.0 %	35.2 %
Sn-I	46.3 %	36.7 %	0.7 %	16.3 %
Sn-II	39.3 %	54.5 %	0.3 %	5.9 %
HCT-116				
Control	71.4 %	13.0 %	2.4 %	13.3 %
RSH	72.4 %	16.7 %	7.3 %	3.6 %
Au-I	11.5 %	3.2 %	0.2 %	85.2 %
Au-II	26.9 %	10.1 %	2.1 %	61.0 %
Sn-I	3.9 %	17.3 %	0.5 %	78.3 %
Sn-II	10.9 %	26.0 %	0.2 %	63.0 %

but also mitochondrial swelling and opens the mitochondrial permeability transition pores (MPTp) leading to the cell death [51].

Incubation with compound **Sn-II** increases in the pool of cells of early apoptosis by 1.5 times compared to the original compound **Sn-I**, but the percentage of late apoptotic cells was 3 times less. After 48 h of incubation HCT-116 cells were more sensitive to the action of the studied compounds. The percentage of late apoptotic cells for tin and gold complexes was higher than 50 %. For **Sn-II** and **Au-II** complexes

containing a phenolic group the number of late apoptotic cells was lower than early apoptotic cells compared to the precursors **Au-I** and **Sn-I**. Thus, for HCT-116 cells the effect of the antioxidant fragment R in **Au-II** and **Sn-II** retards the development of apoptosis. For both cell lines, the percentage of late apoptotic cells in the starting substances was higher than in the complexes, furthermore, the number of cells in the early apoptosis stage was higher for compounds with an antioxidant fragment (Fig. 4).

The effect of compounds in $2 \cdot IC_{50}$ concentration on the cell cycle was studied by mixture of reagents including the nuclear DNA intercalating dye propidium iodide (PI) and RNases in a proprietary formulation after incubation for 24 and 72 h [50].

The technique is based on the determination of DNA content by PI staining to recognize and measure the percentage of cells in each phase of the cell cycle (G0/G1, S and G2/M). PI distinguishes cells at different stages of the cell cycle based on different DNA content in the presence of RNase which increases the specificity of DNA staining. Cells in the G0/G1 phase contain two copies of each chromosome. As cells progress through the cell cycle, they synthesize chromosomal DNA (S-phase). The fluorescence intensity from PI increases until all chromosomal DNA doubles (G2/M phase). At this stage, G2/M cells fluoresce at twice the intensity of the G0/G1 population. G2/M cells divide into two daughter cells at the end of the cycle. The effect of the studied compounds on the cell cycle for MCF-7 and HCT-116 cells is presented in Table 6. For the MCF-7 cell line the effect of the compounds is insignificant. It can be noted that **Au-II** complex presumably blocks G0/G1 phase. For **Au-II** and **Sn-II** complexes decreased the part of cells in G0/G1 phase that seems to be associated with the appearance of dead cells whose DNA is destroyed. The influence of the studied compounds on the cell cycle for HCT-116 cells is presented in Figs. 5 and 6.

Au-I and **Sn-I** do not change essentially the cell cycle of HCT-116 cells. The **Au-II** causes a block at the G0/G1 stage. **RSH** results in a minor G1 phase block after 24 h of incubation. **Sn-II** causes a marked block of the cell cycle in the G2/M phase after 24 and 72 h and initiate cell death. The results demonstrate that the effect on cell cycle depends on compounds and cells type. The pronounced influence was found for HCT-116 cells. High cytotoxicity of **Sn-II** towards MCF7 and HCT-116 cancer cells indicates the perspectives of this compound for preclinical studies.

Mechanisms of Sn and Au cytotoxicity

The possible mechanisms of **Sn-II** cytotoxicity including tubulin binding and influence on tubulin assembly in microtubules was investigated previously [51]. **Sn-II** revealed the significant inhibition of tubulin polymerization without disturbances of microtubules structure (Fig. 7).

Since tubulin is a sulphhydryl-rich protein with 20 cysteine residues distributed across both subunits, it can react with sulphhydryl-directed reagents, the interaction of **Sn-II** with tubulin did not provide

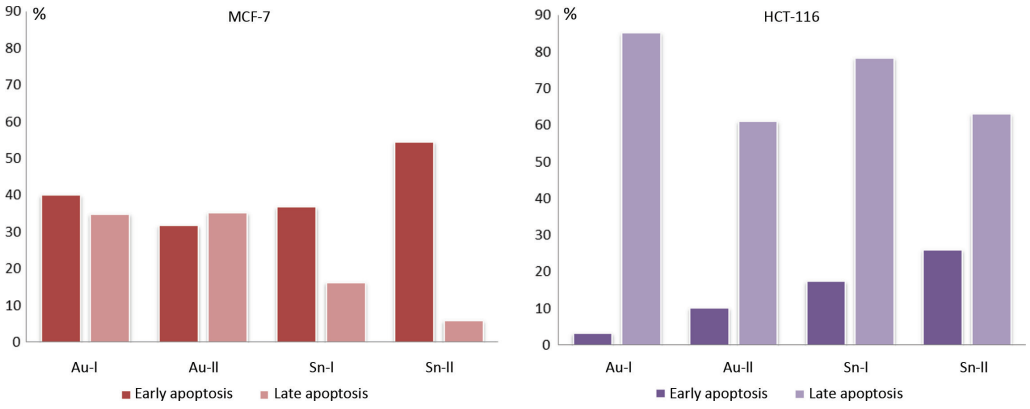


Fig. 4: Percentage of MCF-7 and HCT-116 cells in early and late apoptosis after 48 h of incubation.

Table 6: Effect of compounds on the cell cycle of MCF-7 and HCT-116.

Compound	Incubation time, h	MCF-7			HCT-116		
		G_0/G_1	S	G_2/M	G_0/G_1	S	G_2/M
Control	24	58.9	15.8	20.1	36.0	25.3	22.7
	72	74.3	12.1	11.1	47.8	22.8	21.2
RSH	24	58.6	16.0	19.9	44.6	17.1	26.0
	72	73.6	8.6	12.9	45.1	18.8	23.2
Au-I	24	54.6	19.6	19.9	39.2	27.2	19.7
	72	72.0	10.6	14.3	50.5	24.3	19.5
Au-II	24	65.7	15.8	14.9	43.6	22.7	22.7
	72	49.6	18.3	22.2	52.1	20.5	22.5
Sn-I	24	45.5	15.7	31.3	39.3	24.0	26.7
	72	69.0	13.3	14.5	50.5	20.6	23.2
Sn-II	24	66.1	15.9	12.1	31.7	20.3	31.2
	72	52.4	11.6	14.7	36.8	18.3	32.5

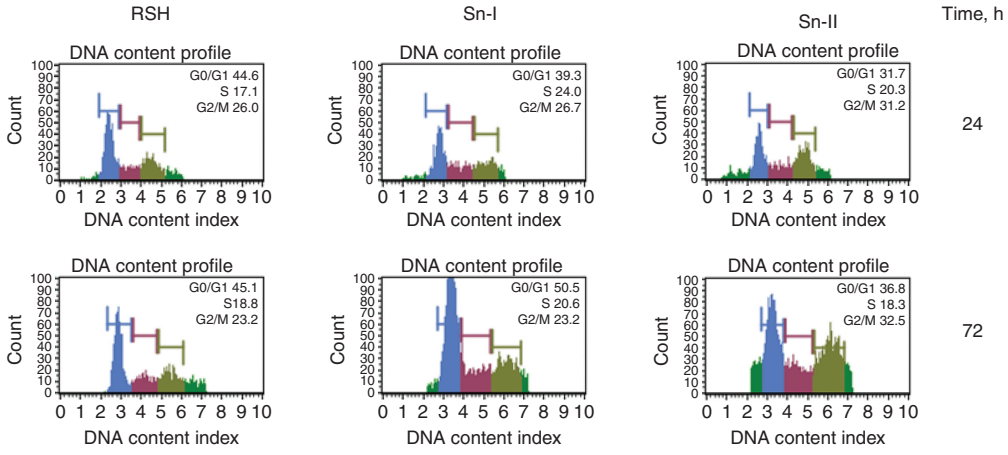


Fig. 5: Cell cycle analysis by flow cytometry in **HCT-116** cancer cells were treated by **RSH, Sn-I** and **Sn-II** for 24 and 72h, concentration of compounds $2 \cdot IC_{50}$.

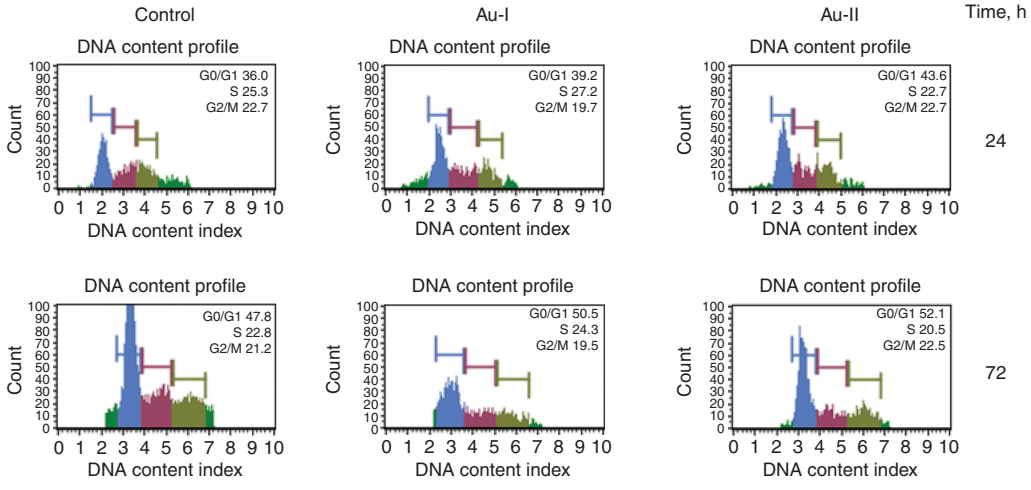


Fig. 6: Cell cycle analysis by flow cytometry in **HCT-116** cancer cells were treated by **Au-I** and **Au-II** for 24 and 72h, concentration of compounds $2 \cdot IC_{50}$.

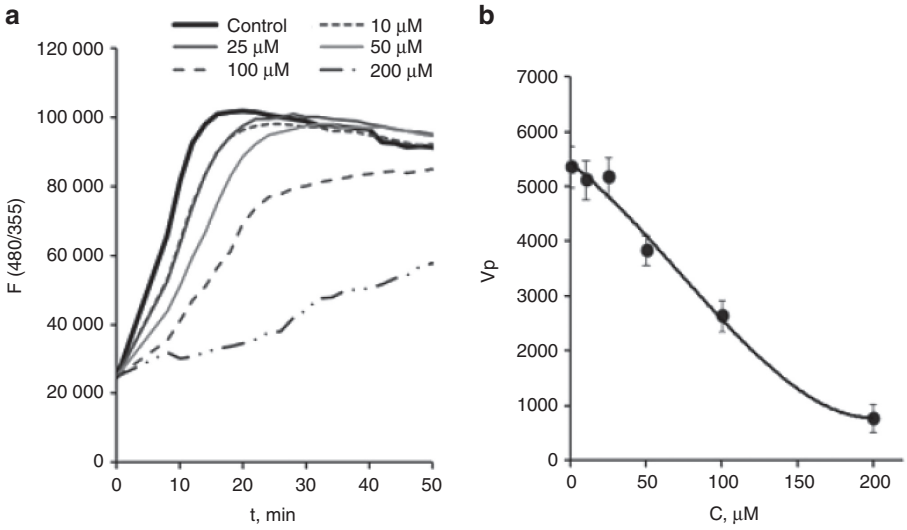


Fig. 7: The influence of **Sn-II** on tubulin polymerization (a) kinetic curve of tubulin polymerization as the change in fluorescence intensity; (b) the concentration dependence of tubulin polymerization rate (Vp) in the presence of compound **Sn-II**.

distinct evidences for the putative binding modes and modes of action of the compounds. For insight of mode of interaction between **Sn-II** and tubulin, docking simulation for this compound towards well-studied colchicine, paclitaxel and vinblastine binding pockets in the tubulin molecule were carried out. The main contribution into the calculated binding energy score of compoundы was provided by van-der-Waals interactions. A large size of the molecule hampers binding with colchicine site. Predicted binding modes of the compound in paclitaxel and vinblastine binding sites are much more reasonable (Fig. 8).

Since organotins form significantly fewer hydrogen bonds than natural compounds, their binding is ruled mostly by shape complementarity. **Sn-II** can overlap with paclitaxel and vinblastine sites, but their totally different shape requires specific binding modes and this compound cannot penetrate into the deepest parts of the pockets.

Thioredoxin reductase (TrxR) – redox enzyme which maintains the balance between pro- and anti-oxidative states in living cells. TrxR as a major target of auranofin been inhibited by auranofin leads to an oxygen stress, cytotoxicity and induced damage of tissues in which auranofin accumulates that complicate the practical use of auranofin. Many attempts have been made to minimize the pro-oxidant effect of auranofin. Modulation of mammalian TrxR was investigated as a tentative drug target. The **Au-II** inhibited TrxR with an IC_{50} value of 0.57 ±0.15 mM. Auranofin showed a higher activity (IC_{50} 0.016 ± 0.003 mM) whereas

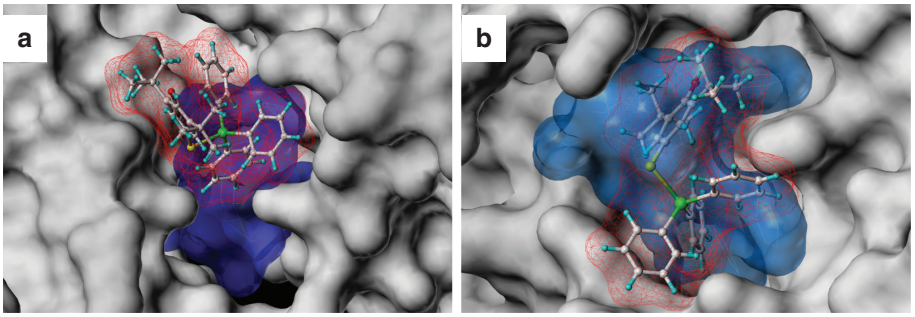


Fig. 8: The best scored orientations of compound **Sn-II** (a, b) in the ligand binding sites of 4EB6 (a) and 1JFF (b). Cognate ligands [vinblastine (a) and paclitaxel (b)] are shown as blue translucent surfaces, organotin is shown as balls-and sticks and red coloured surface [51].

RSH had no effect at up to 10 mM [32]. These results justified our attempts of chemical modification of **Au-I** leading to derivatives with an attenuated prooxidant potency.

Toxicity of Sn and Au compounds *in vivo*

The experiments were performed on white Balb/c mice weighing 18–20 g, age 8–10 weeks. Compounds **Sn-II** and **Au-II** were injected into the abdominal cavity in physiological buffer solution. The volume of injected solutions is 0.5 ml, the volume of solvent (DMSO) does not exceed 125 µl. In the initial experiments, the upper limit of tolerability of administered doses was estimated. Two doses were chosen: 62.5 mg/kg and 125 mg/kg (1.25 mg/mouse and 2.5 mg/mouse, respectively). Each group had 4 animals. After 7 days all mice in both groups (62.5 mg/kg **Sn-II** or **Au-II**) were alive. In **Sn-II** group (125 mg/kg) two mice died on third day. In **Au-II** (125 mg/kg) group all mice died after first day of experiment. The results of the initial evaluation showed that for compounds **Sn-II** and **Au-II**, a dose of 125 mg/kg was lethal. In the following experiments, animals received doses of compounds **Sn-II** and **Au-II** from 70 mg/kg to 110 mg/kg once intraperitoneal injection in each group, 6 mice. Observations were continued for 21 days. Table 7 shows the results of the study of acute general resorptive toxicity of compounds **Sn-II** and **Au-II**. The introduction was accompanied by toxic manifestations (behavioral-stooping, numbness, sometimes inactivity). When administered doses of 70–80 mg/kg, these manifestations lasted from several hours to 1 day, after which the condition of the animals was restored and, in the future, did not differ from that of intact mice. For compounds **Sn-II** and **Au-II**, administration of doses >80 mg/kg was fatal for some animals. Thus, 80 mg/kg is the maximum tolerated dose.

Conclusions

The synthesis of Sn(IV) and Au(I) complexes bearing 2,6-di-*tert*-butylphenol moiety has been accomplished by our research group. The results of studies of Sn(IV) and Au(I) compounds indicate a difference in the modes of compounds action depending on the nature of the metal type and presence of antioxidant fragment. While Ph_3SnCl and Ph_3PAuCl compounds act as prooxidants, complexes **Ph_3SnSR** and **Ph_3PAuSR** based on 2,6-di-*tert*-butyl-4-mercaptophenol (**RSH**) can act as radical scavengers, reducing agents and effective inhibitors of induced mitochondrial lipid peroxidation and provides the transport to biological targets in normal cells. Complexes of Sn exhibit significantly higher cytotoxicity than Au compounds. Tin compounds exhibit an extraordinary antiproliferative properties in different cancer cells and 2,6-di-*tert*-butyl-4-mercaptophenol ligand decreases cytotoxic activity. The introduction of hindered phenol groups decreases the cytotoxicity of complexes. Organotin complex **Ph_3SnSR** depolarizes the mitochondria, induces the mitochondrial permeability transition and causes blocking of the stage preceding mitosis that may be the main reasons of its cytotoxicity. **Ph_3PAuSR** attenuated the potency of the reference drug Auranofin to inhibit TrxR and can acts as a broad specificity organ protector against oxygen burst. Experiments *in vivo* demonstrated that the 5 $mg \cdot kg^{-1}$ daily for 14 days was safe and efficient dose.

Table 7: Survivability of Balb/c mice with intraperitoneal injection of compounds **Sn-II** and **Au-II**.

Compound	Dose, mg/kg				
	70	**80**	**90**	**100**	**110**
Sn-II	0/6	0/6	3/6	5/6	5/6
Au-II	0/6	0/6	3/6	5/6	6/6

The polytopic Sn(IV) and Au(I) compounds combining 2,6-di-*tert*-butylphenol pendant and metal center in one molecule are membrane active compounds and may be studied with the aim to find novel metal-based drugs with low undesirable toxicity.

Acronyms

A498	Renal cell line
A549	Lung cancer cell line
7-AAD	7-Aminoactinomycin D
BHT	2,6-di-*tert*-butyl-4-methylphenol
BGT	2,6-di-*tert*-butyl-4-methoxyphenol
CaCo-2	Colon cell line
CUPRAC	Cupric Ion Reducing Antioxidant Capacity
DCFH-DA	2′,7′-Dichlorofluorescin diacetate
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DMSO	Dimethyl sulfoxide
EC_{50}	Efficient Concentration
FDA	Food and Drug Administration
GFP	Green fluorescent proteins
HCT-116	Human colon cancer cell line
MDA	Malonic dialdehyde
MCF-7	Breast cancer cell line
MCF10A	Conditionally normal cells (breast epithelial cells)
M14	Melanoma
MitoSOX	Mitochondrial Superoxide Indicator
MPTp	Mitochondrial permeability transition pores
NADPH	Nicotinamide adenine dinucleotide phosphate
NCI-H322M	Non-small-cell lung carcinoma cell line
PDT	Photodynamic therapy
PI	Propidium iodide
PS	Phosphatidylserine
ROS	Reactive oxygen species
RNA	Ribonucleic acid
SNB-19	Glioblastoma cell line
TBARs	Thiobarbituric acid reactive substances
TEAC	Trolox equivalents antioxidant capacity
TrxR	Thioredoxin reductase
Trx	Thioredoxin
VA13	Embryonic lung fibroblasts

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References

[1] E. R. Milaeva, V. Y. Tyurin. *Pure Appl. Chem*. **89**, 1065 (2017).
[2] A. Pagliarani, F. Trombetti, V. Ventrella (Eds.), *Biochemical and Biological Effects of Organotins*, Bentham eBooks, Sharjah (2012).
[3] E. R. Milaeva, V. S. Petrosyan, N. T. Berberova, Y. T. Pimenov, L. Pellerito. *Bioinorg. Chem. Appl.* **2**, 69 (2004).
[4] C. N. Banti, S. K. Hadjikakou, T. Sismanoglu, N. Hadjiliadis. *J. Inorg. Biochem.* **194**, 114 (2019).
[5] J. Devi, J. Yadav. *Anti-Cancer Agents Med. Chem.* **18**, 335 (2018).
[6] S. K. Hadjikakou, N. Hadjiliadis. *Coord. Chem. Rev.* **253**, 235 (2009).
[7] M. N. Xanthopoulou, S. K. Hadjikakou, N. Hadjiliadis, M. Schürmann, K. Jurkschat, J. Binolis, S. Karkabounas, K. Charalabopoulos. *Bioinorg. Chem. Appl.* **1**, 227 (2003).
[8] M. N. Xanthopoulou, S. K. Hadjikakou, N. Hadjiliadis, M. Schürmann, K. Jurkschat, A. Michaelides, S. Skoulika, T. Bakas, J. Binolis, S. Karkabounas, K. Charalabopoulos. *J. Inorg. Biochem.* **96**, 425 (2003).
[9] M. A. Abdellah, S. K. Hadjikakou, N. Hadjiliadis, M. Kubicki, T. Bakas, N. Kourkoumelis, Y. V. Simos, S. Karkabounas, M. M. Barsan, I. S. Butler. *Bioinorg. Chem. Appl.* **2009**, 542979 (2009).
[10] V. I. Balas, I. I. Verginadis, G. D. Geromichalos, N. Kourkoumelis, L. Male, M. B. Hursthouse, K. H. Repana, E. Yiannaki, K. Charalabopoulos, T. Bakas, S. K. Hadjikakou. *Eur. J. Med. Chem.* **46**, 2835 (2011).
[11] V. I. Balas, S. K. Hadjikakou, N. Hadjiliadis, N. Kourkoumelis, M. E. Light, M. Hursthouse, A. K. Metsios, S. Karkabounas. *Bioinorg. Chem. Appl.* MS. ID. 654137 (2008).
[12] M. N. Kolyada, V. P. Osipova, N. T. Berberova, Yu. T. Pimenov, E. R. Milaeva. *Macroheterocycles* **10**, 57 (2017).
[13] A. Gennari, B. Viviani, C. L. Galli, M. Marinovich, R. Pieters, E. Corsini. *Toxicol. Appl. Pharmacol.* **169**, 185 (2000).
[14] L. Niu, Y. Li, Q. Li. *Inorg. Chim. Acta* **423**, 2 (2014).
[15] G. K. Latsis, C. N. Banti, N. Kourkoumelis, C. Papatriantafyllopoulou, N. Panagiotou, A. Tasiopoulos, A. Douvalis, A. G. Kalampounias, T. Bakas, S. K. Hadjikakou. *Int. J. Mol. Sci.* **19**, 2055 (2018).
[16] M. N. Xanthopoulou, S. K. Hadjikakou, N. Hadjiliadis, E. R. Milaeva, J. A. Gracheva, V. Y. Tyurin, N. Kourkoumelis, K. C. Christoforidis, A. K. Metsios, S. Karkabounas, K. Charalabopoulos. *Eur. J. Med. Chem.* **43**, 327 (2008).
[17] M. N. Xanthopoulou, S. K. Hadjikakou, H. N. Kourkoumelis, E. R. Milaeva, Y. A. Gracheva, V. Y. Tyurin, I. Verginadis, S. Karkabounas, M. Baril, I. S. Butler. *Russ. Chem. Bull.* **56**, 767 (2007).
[18] M. N. Xanthopoulou, N. Kourkoumelis, S. K. Hadjikakou, N. Hadjiliadis, M. Kubicki, S. Karkabounas, T. Bakas. *Polyhedron* **27**, 3318 (2008).
[19] *Drugs R&D* **5**, 58 (2004). https://doi.org/10.2165/00126839-200405010-00013.
[20] R. Kour Sodhi, S. Paul. *Cancer Ther. Oncol. Int. J.* **14**, MS.ID.555883 (2019).
[21] T. Zou, C. T. Lum, C.-N. Lok, J.-J. Zhanga, C.-M. Che. *Chem. Soc. Rev.* **44**, 8786 (2015).
[22] S. Tian, F.-M. Siu, S. C. F. Kui, C.-N. Lok, C.-M. Che. *Chem. Comm.* **47**, 9318 (2011).
[23] V. Fernández-Moreira, R. P. Herrera, M. C. Gimeno. *Pure Appl. Chem.* **91**, 247 (2019).
[24] C. Roder, M. J. Thomson. *Drugs R&D* **15**, 13 (2015).
[25] A. Casini, L. Messori. *Curr. Top. Med. Chem.* **11**, 2647 (2011).
[26] C. Marzano, V. Gandin, A. Folda, G. Scutari, A. Bindoli, M. P. Rigobello. *Free Rad. Biol. Med.* **42**, 872 (2007).
[27] K. N. Kouroulis, S. K. Hadjikakou, N. Kourkoumelis, M. Kubicki, L. Male, M. Hursthouse, S. Skoulika, A. K. Metsios, V. Y. Tyurin, A. V. Dolganov, E. R. Milaeva, N. Hadjiliadis. *Dalton Trans.* **47**, 10446 (2009).
[28] E. Georgiou, A. Metsios, N. Kourkoumelis, S. Karkabounas, K. Charalabopoulos, A. Badeka, S. K. Hadjikakou. *J. Enzyme Inhib. Med. Chem.* **4**, 592 (2011).
[29] M. P. Chrysouli, C. N. Banti, N. Kourkoumelis, N. Panayiotou, G. S. Markopoulos, A. J. Tasiopoulos, S. K. Hadjikakou. *J. Inorg. Biochem.* **179**, 107 (2018).
[30] F. Radenkovic, O. Holland, J. J. Vanderlelie, A.V. Perkins. *Biochem. Pharmacol.* **146**, 42 (2017).
[31] D. B. Shpakovsky, C. N. Banti, E. M. Mukhatova, Y. A. Gracheva, V. P. Osipova, N. T. Berberova, D. V. Albov, T. A. Antonenko, L. A. Aslanov, E. R. Milaeva, S. K. Hadjikakou. *Dalton Trans.* **43**, 6880 (2014).
[32] D. B. Shpakovsky, A. A. Shtil, E. V. Kharitonashvili, V. Y. Tyurin, T. A. Antonenko, A. A. Nazarov, V. P. Osipova, N. T. Berberova, L. S. Foteeva, C. Schmidt, I. Ott, E. R. Milaeva. *Metallomics* **10**, 406 (2018).
[33] E. Denisov (Ed.), *Handbook of Antioxidants*, CRC Press, Boca Raton (2018).
[34] R. Amorati, A. Baschieri, L. Valgimigli. *J. Chem.* MS. ID. 6369358 (2017).
[35] Z. Huyut, S. Beydemir, I. Gulcin. *Biochem. Res. Int.* MS. ID. 7616791 (2017).
[36] I. Belostotskaja, E. Burlakova, V. Misin, G. Nikiforov, N. Khrapova, V. Shtol'ko. *Chem. Chem. Technol.* **7**, 457 (2013).
[37] D. B. Shpakovsky, C. N. Banti, G. Beaulieu-Houle, N. Kourkoumelis, M. Manoli, M. J. Manos, A. J. Tasiopoulos, S. K. Hadjikakou, E. R. Milaeva, K. Charalabopoulos, T. Bakas, I. S. Butler, N. Hadjiliadis. *Dalton Trans.* **41**, 14568 (2012).
[38] E. R. Milaeva. *Curr. Top. Med. Chem.* **11**, 2703 (2011).
[39] C. Pellerito, L. Nagy, L. Pellerito, A. Szorcsik. *J. Organomet. Chem.* **691**, 1733 (2006).
[40] E. R. Milaeva, D. B. Shpakovsky, V. P. Dyadchenko, A. I. Gryzlov, Yu. A. Gracheva, T. A. Antonenko, M. J. Parulava, D. V. Albov, L. A. Aslanov, L. G. Dubova, P. N. Shevtsov, M. E. Neganova, E. F. Shevtsova. *Polyhedron* **127**, 512 (2017).

[41] R. Apak, K. Guclu, M. Ozyurek, S. E. Karademir. *J. Agric. Food Chem.* **52**, 7970 (2004).
[42] T. A. Antonenko, D. B. Shpakovsky, M. A. Vorobyov, Y. A. Gracheva, E. V. Kharitonashvili, L. G. Dubova, E. F. Shevtsova, V. A. Tafeenko, L. A. Aslanov, A. G. Iksanova, Y. G. Shtyrlin, E. R. Milaeva. *Appl. Organomet. Chem.* **32**, e4381 (2018).
[43] W. Brand-Williams, M. E. Cuvelier, C. Berset. *Food Sci. Technol.* **28**, 25 (1995).
[44] I. Kubo, N. Masuoka, T. J. Ha, K. Tsujimoto. *Food Chem.* **99**, 555 (2006).
[45] Y. C. Chung, S. J. Chen, C. K. Hsu, C. T. Chang, S. T. Chou. *Food Chem.* **91**, 419 (2005).
[46] I. Gulcin, Z. Huyut, M. Elmastas, H. Y. Aboul-Enein. *Arab. J. Chem.* **3**, 43 (2010).
[47] M. E. Neganova, V. A. Blik, S. G. Klochkov, N. E. Chepurnova, E. F. Shevtsova. *Neurochem. J.* **5**, 208 (2011).
[48] V. S. Petrosyan, Y. A. Gracheva, V. Y. Tyurin, E. V. Grigor'ev, E. R. Milaeva, L. Pellerito. *Russ. J. Org. Chem.* **39**, 353 (2003).
[49] S.-H. Lee, X. W. Meng, K. S. Flatten, D. A. Loegering, S. H. Kaufmann. *Cell Death Differ.* **20**, 64 (2012).
[50] A. Dziedzic, R. Kubina, A. Kabała-Dzik, M. Tanasiewicz. *Evid. Based Complement Alternat Med.* MS. ID. 6793456 (2017).
[51] E. R. Milaeva, D. B. Shpakovsky, Yu. A. Gracheva, T. A. Antonenko, D. I. Osolodkin, V. A. Palyulin, P. N. Shevtsov, M. E. Neganova, D. V. Vinogradova, E. F. Shevtsova. *J. Organomet. Chem.* **782**, 96 (2015).

Graphical abstract

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Novel selective anticancer agents based on Sn and Au complexes. Mini-review

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Pure Appl. Chem. 2020; x(x): xxx–xxx

Conference paper: Cytotoxic and antioxidant properties *in vitro* and *in vivo* of organotin and gold complexes based on 2,6-di-*tert*-butyl-4-mercaptophenol is discussed

Keywords: 2,6-di-*tert*-butylphenol; antioxidants; apoptosis; Au(I) complexes; cell cycle; cytotoxicity; Mendeleev-21; metal-based drugs; organotin compounds; toxicity *in vivo*.