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# Innovative gold complexes with CN group as anticancer agents – possible mechanisms of action

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## ABSTRACT

Several gold(I) and gold(III) compounds described so far have significant antiproliferative effects *in vitro* against selected tumor cell lines. Gold (III) has the same electronic configuration as platinum(II); moreover, some gold(III) complexes have the same square-planar structure as cisplatin. In addition, the compounds are water-soluble and display low toxicity. The disadvantage of gold(III) derivatives is low stability in physiological conditions that reduces their applications. Thus, an invention of novel gold complexes and characterization of their mechanisms of action is extremely urgent. Here, we invented and synthetized a library of innovative gold(III)-based drug-candidates. Those metallodrugs were synthetized according to the following formula: $[Au\ (CN)_n]_m^{(m-p)n} \cdot (ClO_4)_m$, where: "m" is a number from 3 to 6, "n" is from 1 to 10, "p" is from 1 to 3 and "r" is from 0.1 to 2. Initial physicochemical studies showed that novel gold(III) complexes were stable in water, blood and lymph. Within this work, our team aimed to critically discuss the mechanisms of action of new gold(III) complexes in cancer treatment, in particular the complex TGS121: $[Au(CN)_4]_2(ClO_2)Na$, based on the structure of the new compound and available experimental evidence. Great antiproliferative properties were observed against Ha-Ras-transfected malignant fibroblasts. The IC50 values for parental normal fibroblasts were significantly lower than in transfected cells. TGS121 turned out to be high selective for malignant cells in comparison to normal cells. The analysis of the mole-cular structure suggested that TGS121 could produce a biological effect through a variety of molecular mechanisms. These results make the novel gold(III) complex attractive for further evaluation as an anticancer agent.

Keywords: bioorganometallics; biphenyl; cancer; gold chelates; gold (III) complexes, TGS121

## INTRODUCTION

Inorganic chemistry parallel to organic chemistry is beginning to have an increasing impact on medicine. Cisplatin, a platinum(II) complex, was the first metal-based agent to enter into worldwide clinical use for the treatment of cancer. The discovery of the anticancer effects of cisplatin, around 1965 (Rosenberg, 1985), suggested that platinum and non-platinum metal-based compounds might complement the role of organic anti-cancer drugs (Galluzzi, 2014). This successful platinum-based complex is effective at inhibiting the activity of cancer cells by producing a direct lesion on DNA. This non-selective, DNA-targeting mechanism produces several side effects, such as cardiotoxicity, nephrotoxicity and neurotoxicity (Rezaee 2017; Wang, 2013; Altoum, 2017). Currently other antitumor, metal-based complexes are studied, like ruthenium, gold and titanium (Muhammad, 2014). Amongst them, complexes of gold in oxidation states +I and +III have attracted particular attention. Several studies highlighted that the binding affinity of gold complexes for the DNA was relatively low, indicating that gold compounds have a different mechanism of action than cisplatin (Casini, 2008; Casini, 2010; Mirabelli, 1986). In 1985, auranofin, a gold(I) complex, was approved by the U.S. Food and Drug Administration (FDA) as a therapeutic agent to treat rheumatoid arthritis. Moreover, auranofin demonstrated promising results for the treatment of various malignancies including: leukemia, lung, ovarian, gastric and pancreas cancer 9 (Furst, 1983; Fiskus, 2014; Park, 2014; Zou, 2015; Rios Perez, 2019; Xiaobo, 2016). Concurrently, gold(III) compounds, due to the same squareplanar structure to cisplatin and the fact that gold in oxidation states +III is isoelectronic to platinum in +II, have been qualified as excellent candidates for potential anticancer drugs. The oxidation state +III typically bears a pronounced oxidizing character, unless the gold(III) center is stabilized by an appropriate set of ligands (Gabbiani, 2011). Thus, clinical usefulness of gold(III) compounds was found to be limited, because Au(III) was reduced to

Au(I) or even Au(0) under physiological conditions. This disadvantage slowed down the investigation of gold(III) derivatives in anti-cancer treatment. In mid-1990, Parish et al. (Parish, 1996) invented the first gold(III) complexes with acceptable solution stability and with encouraging results of *in vitro* cytotoxicity toward selected human tumor cell lines. Subsequently, several other classes of cytotoxic gold(III) compounds were developed in many laboratories all over the world, but none of them have been approved for clinical use (Casini, 2009).

Some initial indications concerning the possible mechanisms of action of gold(III) compounds were obtained. As mentioned above, it seems unlikely that all gold compounds work analogously to cisplatin. In fact, interactions with proteins may play key roles in the mechanism of action and in the toxic effects of these antitumor metal complexes (Mirabelli, 1986; Gabbiani, 2011). Nevertheless, the molecular targets of antiproliferative gold compounds are still largely unknown and a subject of intense research and debate. Casini et al. (Casini, 2009) evaluated the anticancer properties of a group of gold(III) derivatives against a panel of 36 human tumor cell lines using a systematic screening strategy. It was observed that the antiproliferative properties essentially rely on a variety of distinct molecular mechanisms. In particular, possible targets for the investigated gold compounds were proposed, e.g., thioredoxin reductase, protein kinase C, histone deacetylase, and proteasome. On the other hand, Barnard et al. (Barnard, 2007) advanced a hypothesis that cytotoxic gold compounds, in particular gold(I) compounds, produce their biological effects mainly through a direct antimitochondrial mechanism. No doubt, the same mechanism cannot be excluded for gold in oxidation states +III.

Analysis of the state of the art in the area of metallodrugs in anticancer treatment, particularly based on gold, clearly shows that there is still room for improvement and strongly encourages further exploration of the field. An ideal gold-based chemotherapeutic agent should be well-soluble in water, stable in the blood and lymph and efficient at a relatively low dose. The biggest disadvantage in the clinical application of currently available gold complexes is their breakdown in the blood and lymph, decreasing the therapeutic efficacy. This adverse appearance leads to the requirement of the use of much higher doses, which in turn causes increased risk of gold accumulation in selected organs and side effects. Consequently, four new, innovative gold(III)-based drugs were synthesized by the following formula: $[Au\ (CN)_n]_m^{(m-p)n} \cdot (ClO_4)_m$, where: "m" is a number from 3 to 6, "n" is from 1 to 10, "p" is from 1 to 3 and "r" is from 0.1 to 2. Here, we describe the composition and properties of one compound, termed TGS121, synthetized using innovative mono ion technology. This novel method results in elevated bioavailability through the prevention of formation of large water-soluble gold clusters. These clusters are characterized by the presence of a metal – metal bond (Au-Au) and have the structure of crystallographic lattices that are not able to freely pass through the cell membrane. The novel gold(III) complex has no Au – Au bonds, which provides better delivery and can reduce the compound dosage in biological applications. Initial physicochemical studies showed that the novel gold(III) complex was highly watersoluble, stable in water, blood and lymph, and impervious to sunlight. Furthermore, TGS121 was tested for anti-inflammatory properties *in vitro* and *in vivo* (Krajewska, 2021). The obtained results showed significant anti-inflammatory activity in colitis and gave a very solid basis for further preclinical investigations of this gold(III) complex.

Our distant goal is to examine the anticancer potential of new gold(III) derivatives. In this study – as an introduction to the consecutive investigation – we attempted to define the most probable molecular targets for the novel gold(III) complex TGS121. We propose that this compound may be a good candidate for further assessment of its safety and utility as a potential antitumor drug.

## MATERIALS AND METHODS

### SYNTHESIS OF TGS121

The chlorite-cyanide complex of monoionic gold (III) was prepared as described by Krajewska et al. *(*Krajewska, 2021). Briefly:

Pure metallic gold was dissolved in a mixture of concentrated hydrochloric and nitric acid in a molar ratio of 3:1 Next step was: heating with concentrated HCl, followed by removing liquid

acids from the gold (III) salt until the dry gold salt was obtained. The product was dissolved in aqua regia – in order to obtain clusters of gold (III) chloride smaller than 11-atom. Then, small gold clusters were treated once again with 6M HCl – to obtain an orange-red salt of gold (III) chloride, the analysis of which showed the presence of practically pure $Au_2Cl_6$. The product was treated with NaCl in a presence of water that lead to formation of a compound with the formula $Na_2Au_2Cl_8$. Next, the salt was treated with 6N HCl to obtain a solution of $HAuCl_2*H_2O$ monatomic gold salt with a pH of approximately 1.0. In the next step, the solution was neutralized by NaOH to pH 4-5 followed by the addition of $NaClO_2$ until a stable complex of gold (III) with chlorine dioxide and sodium chloride was obtained, with the formula: $NaAuCl_4* ClO_2*(NaCl)z$, where z is a number over 30.

The last step after neutralization with NaOH to pH 7.8, comprised of treatment with alcoholic sodium cyanide solution until end product was obtained. Recently, well-soluble in water complexes of mono-ionic gold (III) were neutralized to pH 7.4 with 0.1 M NaOH. Next, redistilled water was added to the volume of 1 $dm^3$.

The subject of synthesis was termed TGS121 and was diluted tenfold with saline (9 $g/dm^3$ NaCl) The chemical formula of TGS121: $[Au(CN)_4]_2*(ClO_2)Na$ and a diagram illustrating the composition of the molecule is shown in figure 1.

## CELL CULTURE

For experiments we used parental normal fibroblasts NIH3T3 (ATCC) and their variant with Ha-Ras mutation – named Ras-3T3. The NIH3T3 cell line is a nonmalignant murine fibroblast cell line derived from NIH Swiss mouse embryo culture. The Ras-3T3 cell line was provided by Dr. H. Maruta (Ludwig Institute for Cancer Research, Victoria, Australia). The Ras-3T3 cells were obtained as described previously (Maruta, 1991). Briefly, normal NIH-3T3 fibroblasts were transfected with the v-Ha-ras oncogene inserted into the mammalian retroviral vector pMV7, leading to tumorigenic Ras-3T3 cell line.

Fibroblast cell lines were cultured in Dulbeco (Biochrom, Berlin, Germany) supplemented with 10% heat-inactivated FCS, penicillin (100 U/ml), and streptomycin (100 μg/ml) (all from Sigma-Aldrich). Cells were kept in 25 $cm^2$ tissue flasks (Greiner, Berlin, Germany) in a humidified atmosphere containing 5% $CO_2$ and passaged every 2-3 days.

## VIABILITY ASSAYS

Cells were treated for 24 or 48 h with increasing concentrations of TGS121. Relative cell viability was achieved by means of PrestoBlue Assay (Promega Corporation Madison WI) according to manufacturer's instructions. Absorbance of the experimental solution was measured directly in plates using OmegaStar Fluorescence reader at 550 nM.

The cytotoxic/cytostatic effects of novel compounds on culture cells were examined *in vitro* using PrestoBlue assay (Invitrogen, Carlsbad, CA), as previously described (Strus, 2021). Briefly, cells (5 x $10^3$ cells/well) were seeded in 96-well microtiter plates (BD, Biosciences, San Jose, California, USA) and incubated with serial dilutions of TGS121. TGS121 was added in quadruplicate to a final volume of 200 μL. Appropriate volumes of culture medium were added as controls. After an incubation period of 24 or 48 hours, cells were stained with 25 μl PrestoBlue ready solution for 20 min, according to manufacturer's instructions. Fluorescence of experimental solution was measured directly in plates using OmegaStar Fluorescence reader (BMG LABTECH, Ortenberg, Germany) at 560/590 nm excitation/emission, respectively. Cytostatic/cytotoxic effect was expressed as relative viability of treated cells (% of control cells incubated with medium only) and was calculated as follows: relative viability = (Ae - Ab) x 100/(Ac - Ab), where Ab is background absorbance, Ae is experimental absorbance and Ac is the absorbance of untreated controls.

# RESULTS

## THE STRUCTURE OF NOVEL GOLD COMPLEX TGS121 AND ITS POSSIBLE INTERACTIONS

The obtained gold(III) complex with the formula $[Au(CN)_4]_2$ $(ClO_2)Na$ is a sodium salt of chloride dioxide associated with gold(III)-cyanide group complex (fig. 1). Particular chemical groups and molecules comprising the TGS121 complex ale displayed in figure 1. The figure also contains marking of the most evident relations between com-pound's composition and

possible chemical interactions with cellular targets. Relatively low molecular mass (692.5 g/mol) and the form of sodium salt make this compound well water soluble and stable in physiological fluids like a serum. Moreover, such a molecule can bind to albumins and in this way it can be also transported in living organisms. Chloride dioxide is a free radical containing one electron at incompletely-filled antibonding orbital. The presence of such unpaired electron makes this free radical actively interacting with various biologically active molecules, especially with thioredoxin complex. Eight cyanide groups can interact with thioredoxin, as well as with oxygene chain (complex). The presence of two Au molecules can result in interaction with DNA and with active places of some kinases, like MEK/ERK, PKC.

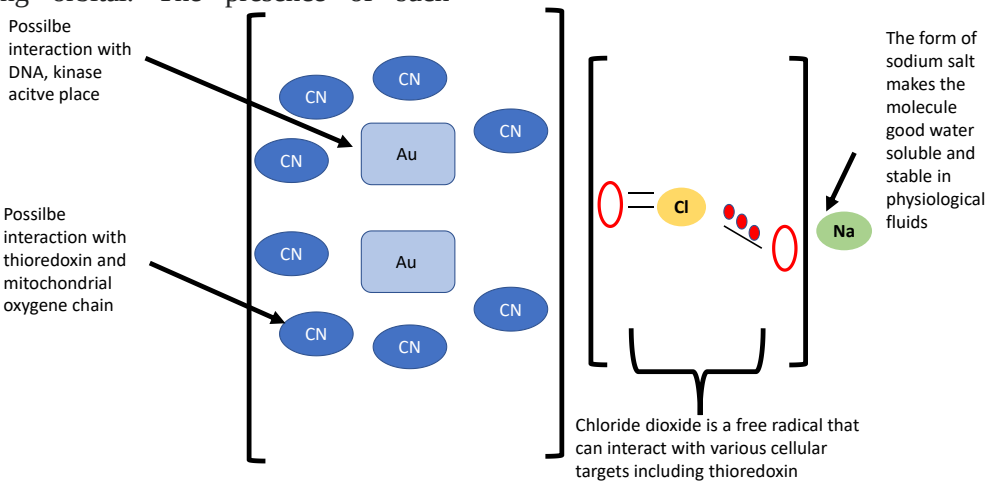


Figure 1. Molecular elements comprising the novel TGS121 complex. Arrows indicate elements possibly interacting with molecular targets in cells

## TGS121 SELECTIVELY INDUCES DEATH OF RAS-3T3 TRANSFECTED FIBROBLASTS

The perfect anticancer drug should kill malignant cells selectively and leave non-tumor cells without damage. To check if TGS121 represents that case, we examined parental normal fibroblasts and its malignant variant with Ha-Ras oncogene hyperactivated. In Ras-3T3 neoplastic cells, the compound TGS121 induced concentration- and time-dependent effect with IC50 at 87.5 ug/L and 160 ug/L, for 24h and 48h, respectively. For non-malignant NIH3T3 cells, respective IC50 values were considerably higher (tab. 1).

Table 1. Inhibitory Concentrations of 50% (IC50) for TGS121 in malignant and non-malignant mouse fibroblasts indicate that higher TGS121 concentrations are needed for non-malignant NIH3T3 cells to inhibit cell growth of 50%

| | Cell line | Time of incubation (h) | |
|---|---|---|---|
| | | 24 | 48 |
| IC50 (µg/L) for gold(III) complex TGS121 | Ras-3T3 | 87.5 | 160 |
| | NIH3T3 | 350 | 3500 |

Consequently, we calculated the selectivity index for both incubation times. Our calculations showed that the novel gold complex is selective for Ha-Ras transfected malignant cells (tab. 2).

Table 2. The tumor-selectivity index (TS) was calculated by the following equation: TS = mean IC50 in normal cells/mean IC50 in malignant cells

| | Compound | Time of incubation (h) | |
|---|---|---|---|
| | | 24 | 48 |
| Selectivity index | TGS121 | 4 | 21.9 |

## DISCUSSION

### POSSIBLE APPLICATIONS OF TGS121

We obtained novel gold(III) complex TGS121 and studied its antitumor activity in Ha-Ras transfected fibroblasts in comparison to the parental non-malignant NIH-3T3 fibroblasts. The novel TGS121 compound turned out to be active against malignant cells at low doses (ng/mL), its effect was time- and dose-dependent. Low effective doses of the drug may mean low toxicity to healthy cells. TGS121 was not only active for Ha-Ras transfected malignant cells, but also was less toxic for non-malignant fibroblasts. The selectivity index was 21 at 48 h (tab. 2) indicating twenty times less toxicity for normal cells. It may be surprising that the selectivity index after 24 hours is only 4. But this short-time effect on normal cells may be only cytostatic effect. Normal cells may overcome cell death by transient cell cycle arrest, that is impossible for unhampered dividing malignant cells that are destinated for death due to accumulation of errors. Such protective effect was recently shown for podophyllotoxin complex in context of normal human keratinocytes (Strus 2021).

Because deregulated RAS/RAF/MEK pathway drives uncontrolled divisions of tumor cells, drugs selectively targeting cells with Ras hyperactivation can be widely used in cancer treatment (Khojasteh, 2021; Sugiura, 2021; Feleszko, 2000).

In particular three isoforms of Ras protein are recognized: Ha-Ras, N-Ras, Ki-Ras. The protein sequence is 80% identical between them, however, oncogenic mutations of the different isoforms predominate in different tumors. For example, Ha-Ras mutations are found in carcinomas of the bladder, kidney, and thyroid; N-Ras mutations are found in myeloid and lymphoid disorders, liver carcinoma, and melanoma; whereas Ki-Ras mutations predominate in colon and pancreatic carcinoma (Zhang, 1997).

Such oncogenic Ras mutations have been found in about 40% of human cancers and are thought to be a critical factor in the proliferation of these tumors. Thus, TGS121 is promising drug candidate for cancer treatment. Moreover, its physicopharmacological properties also confirm the possibility of using it directly as a drug. Notably, TGS121 complex is water soluble, its solution is stable in neutral pH, is stable at room temperature, the solution is clear and don't form any debris even if stored for several months, we can assume this compound can be given systemically in a form of intravenous infusion. For proper indication of novel TGS121 more studies are needed, extended cell studies, followed by preclinical and clinical ones. Especially there is needed analysis of TGS121 activity in human tumors with known Ha-Ras mutations (like bladder, kidney and thyroid cancer), but also in malignancies with other Ras mutations, since all Ras isoforms share 80% of identity.

### POSSIBLE MECHANISMS OF ACTION OF TGS121 IN COMPARISON TO OTHER GOLD(III) COMPLEXES

#### DIRECT DNA DAMAGE

The model anticancer metallodrug is represented by various platinum complexes, like cisplatin or oxaliplatin, which target DNA (Dasari, 2014). Due to the isoelectronicity and isostructurality of gold(III) compounds to cisplatin, many scientists proposed DNA as the first target of these metallodrugs (Crooke, 1981). Hadjiliadis et al. *(*Hadjiliadis, 1981) proved that $HAuCl_4$, a gold(III) complex, can react with nucleosides. Another study showed that a number of gold(III) complexes may interact with DNA, although by a different chemical mechanism than cisplatin, while some were shown to exhibit an analogous to cisplatin mode of action, but significantly weaker (Mirabelli, 1986). In the same study, investigators established that coordination ligands are a defining factor of gold-DNA reactivity and it was revealed that only gold complexes containing halogen or pseudohalogen ligands interact with DNA. Based on these results researchers suggested increased lability of the gold-halogen to gold-carbon bonds (Mirabelli, 1986).

DNA binding affinity studies performed on several gold(III) porphyrins using purified calf-thymus DNA indicated that the DNA and gold(III) porphyrin interaction appeared to be noncovalent and reversible (Kang, 2005). Wang et al. (Wang, 2007) determined the nature of the reaction between gold(III) porphyrin 1a and DNA. Their results showed that the investigated agent causes fragmentation of DNA *in vivo*,

rather than a connection of two purine bases on the same strand of DNA, like cisplatin. In case of gold porphyrins, the biological activity strongly depended on the nature of the ligand. The porphyrin ligand significantly reduced the redox reactivity and stabilized the gold(III) center (Che, 2003).

A cisplatin-like, DNA cross-linking mechanism was observed in gold(III) dithiocarbamate derivatives (Ronconi, 2006). Importantly, the cytotoxicity of the investigated potential drugs was higher than cisplatin, even if the long term stability of gold(III) – DNA adducts seemed to be low. Moreover, DNA cross-links were repaired less efficiently compared to those induced by cisplatin. According to the obtained results, despite some similarities, the mechanism of action is different from that of cisplatin.

For other gold(III) complexes, weak and reversible interactions with calfthymus DNA were observed in mononuclear bipyridyl gold(III) complexes (Marcon, 2002), polyamine complexes (Messori, 2001), and dinuclear oxo gold(III) complexes bearing bipyridyl ligands (Casini, 2006), whereas tight bonds to DNA were reported in chloroglycyl-histidine gold(III) compounds (Carotti, 2000).

The novel TGS121 compound – due to the presence of a gold(III) atom that is surrounded by small cyano groups can fit into the DNA groove and associate with DNA, with subsequent DNA damage by a free electron donated by the chloride dioxide group (fig. 1).

## THIOREDOXIN REDUCTASE (TRXR)

Multiple cellular processes involve redox-sensitive signaling factors. The thioredoxin system is an essential component in many redox-regulated pathways. It consists of thioredoxin (Trx) and thioredoxin reductase (TrxR). Both proteins have two isoforms, namely cytosolic (Trx1; TrxR1) and mitochondrial (Trx2; TrxR2) (Holmgren, 1985). Thioredoxin reductase is a selenoprotein with a selenocysteine-containing active site (–Gly–Cys–Sec–Gly). Its main function is to maintain Trx in the reduced state and allow donation of electrons to disulfides in proteins (Arnér, 1989). Among Trx substrates, ribonucle-otide reductase (RR)( Holmgren, 1989), NF-kB, AP-1, p53 (Qin, 1995; Abate, 1990; Ueno, 1999), glucocorticoid receptor (Grippo, 1983), ASK1 (Saitoh, 1998), protein kinases C (Watson, 1999) and tumor suppressor PTEN (Meuillet, 2004) have been distinguished.

Gold(III) compounds are known to strongly and selectively target thiol, imidazole and selenol groups of proteins (Casini, 2008). TrxR has a selenol group within the active site, thus it was considered as a good target for gold(III) derivatives. This view is supported by Gromer et al. (Gromer, 1998) who showed that glutathione reductase, an enzyme structurally and functionally similar to TrxR, but devoid of selenium, is significantly less sensitive to inhibition by gold complexes (Gromer, 1998). An increased level of Trx and TrxR has been observed in a number of human tumors, including colorectal cancer (Raffel, 2003). The same authors showed a positive correlation between ele-vated Trx and TrxR levels and increased cell proliferation, implying that the thiore-doxin system may play a crucial role in tumor progression (Grogan, 2000). Therefore, TrxR could be considered as a possible target for gold(III) complexes, similarly to auranofin and some auranofin-like gold(I) compounds (Rigobello, 2004; Marzano, 2007).

Coronnello et al. described a series of organo-gold(III) compounds with excellent anti-proliferative properties on the A2780 ovarian carcinoma cell line. Notably, the examined agents selectively inhibited TrxR. The observed proapoptotic potential of organo-gold(III) compounds most likely resulted from direct interference with mitochondrial functions.

In search of the properties of gold(III)-based TrxR inhibitors, Engman et al. (Engman, 2006) evaluated the effect of the number of carbon-gold bonds in these complexes on their toxicity. Among complexes with none, one, two or three such bonds, complexes with up to two carbon-gold bonds were the most potent TrxR inhibitors. The inhibitory concentration of the studied compounds was insufficient to kill cells.

Summarizing, the optimal structure of gold(III) compound, as an inhibitor of TrxR, consists of two carbon-gold bonds and one exchangeable group that could interact with ligands (Engman, 2006).

Similarly, the novel gold(III) complex TGS121 contains eight cyano groups, which can form at least two carbon-gold bonds; the other cyano groups interact with thioredoxins as ligands.

## PROTEIN KINASE C (PKC)

Protein kinase C (PKC) is a protein kinase involved in cellular proliferation, cell cycle control, differentiation, migration, and survival. Aberrant PKC expression, activity or localization has been observed in various malignant processes. Thus, inhibition of PKC can be a potential therapeutic strategy in cancer treatment. The PKC family, which consists of 15 isoforms, is subdivided into three groups: classical (conventional), novel and atypical. The difference between particular isoforms of PKC depends on their secondary messenger requirements. Therapeutic targets have been developed for several PKC isoenzymes and some have been examined in clinical trials. For instance, the atypical protein kinase C iota (PKCi), unlike other PKCs, does not depend on calcium, diacylglycerol and phospholipid, but may be regulated by 3-phosphoinositides or through other specific protein-protein interactions. PKCi was found to be targeted by some gold derivatives and two compounds, aurothioglucose and aurothiomalate, were shown to inhibit its activation. These compounds were found to inhibit the proliferation of cancer cells, including non-small cell lung carcinoma (NSCLC) (Nobili, 1986).

The formation of gold – PKC kinase adducts is also possible for the TGS121 complex. Moreover, the gold atom in the TGS121 can specifically target the active site of PKC or the free electron of the chloride dioxide moiety can affect signal transduction to downstream elements.

## MAPK – ERK PATHWAY

The extracellular signal-regulated kinase (ERK1/2) pathway is an important signaling component of the cell. This protein cascade regulates many cellular processes, including proliferation and differentiation. Stably activated Ras/Raf/MEK/ERK pathway is responsible for progression in most human cancers. Surprisingly, there also exist data suggesting that ERK plays a crucial role in the regulation of apoptosis. The transient ERK activation stimulates cell proliferation, while longterm ERK activation rather induces apoptosis. Thus, prolonged ERK activation may induce cell death through the intrinsic or extrinsic apoptotic pathway. The proposed mitochondrial-dependent mechanisms include the up-regulation of Bax and p53, as well as the suppression of survival signaling associated with Akt (Zhuang, 2006). The main differentiating factor in this signaling pathway, producing either cell proliferation or programmed cell death, is the duration of ERK activation. Prolonged activation of ERK can lead to programmed cell death through FADD-independent caspase 8 activation (Cagnol, 2006).

Yamagishi et al. confirmed that ERK plays a crucial role in the execution phase of apoptosis. Moreover, they described the possible mechanism of ERK activation by the ASK1-p38 MAPK pathway (Yamagishi, 2005). Hsieh and Papaconstantinou (Hsieh, 2006) suggested that the ASK1-p38 MAPK pathway is regulated through the level of reactive oxygen species (ROS). Reduced thioredoxin has the ability to bind ASK1 and inhibit the function of this protein. However, after Trx oxidation, ASK1 is released in the active form. When the level of ROS is increased, ASK1 is not inhibited by Trx and can activate p38 MAPK, which leads to apoptosis.

Therefore, there is increasing research regarding the influence of gold-based agents on the ERK pathway. Some gold(III) complexes were shown to activate ERK in a pro-longed manner, as long as cells were incubated with the examined gold complex. This mechanism was found for gold(III)-dithiocarbamato complexes, which have been shown to increase the level of phosphorylated ERK1/2 in HeLa cells (Saggioro, 2007). Results of this study suggested that the investigated complexes can inhibit TrxR, which leads to an increased concentration of oxidized Trx. A consequence of this phenomenon is accumulation of hydrogen peroxide. As mentioned in the paragraph above, the lack of reduced Trx does not inhibit the ASK–p38MAPK–ERK1/2 cascade, which promotes apoptosis (fig. 2). Also, hydrogen peroxide accumulation has been shown to cause ERK1/2 phosphorylation, thereby enhancing the apoptotic effect (McCubrey, 2006).

There is also an opposite hypothesis about ERK involvement in apoptosis that is induced by gold complexes. Park et al. *(*Park, 2006) reported that gold(I) compounds lead to apoptosis by activation of p38MAPK, whereas activation of ERK is independent of the concentration of the agent. Moreover, gold(III) porphyrin 1a was shown to

cause cell cycle arrest at the G2-M and G0-G1 phases, as well as increase accumulation of p53 (Wang, 2007). Subsequent results proved that phosphorylation of p38MAPK, induced by the gold compound, was involved in the cell death process (Wang, 2008).

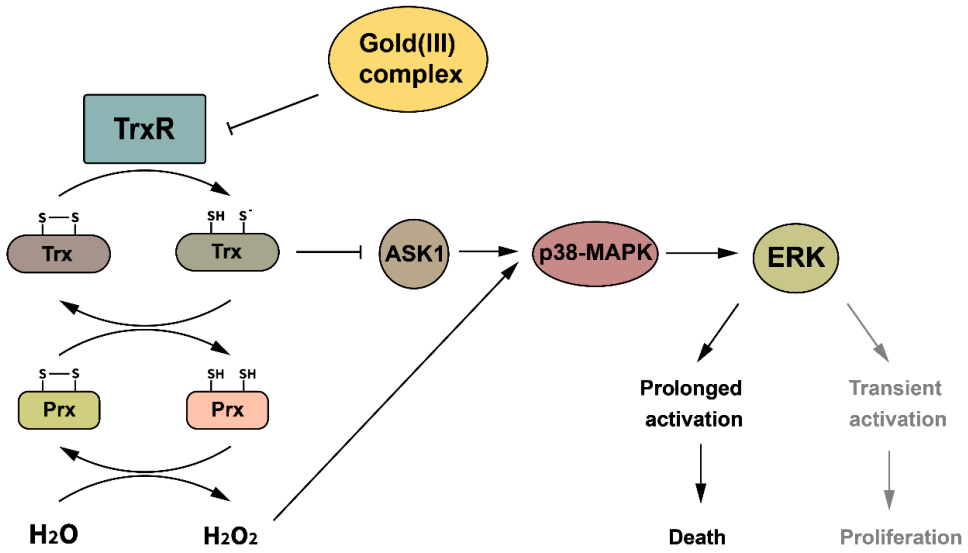


Figure 2. A proposed model for the role of ERK in apoptosis induced by the gold(III) complex

## PROTEASOME

As the ubiquitin–proteasome pathway is essential for cell cycle regulation, apoptosis, angiogenesis and differentiation, it has recently been investigated as an intracellular target for gold(III) compound–induced cytotoxicity. Some gold complexes like the gold(III) dithiocarbamate compound were shown to inhibit proteasome activity and induce accumulation of poly-ubiquitin complexes, both *in vitro* in tumor cell lines, as well as in xenografts resected from experimental animals (Arsenijević, 2012).

These data suggest that proteasomes may be a target for gold(III) complexes and confirm that inhibition of the proteasomal activity could be one of the mechanisms of action of these compounds, including TGS121.

## CONCLUSIONS

The novel compound TGS121 can exert multimodal effects in cells. These effects arise from the structure of TGS121, as it is a water soluble, relatively small molecule, containing various active groups. Thus, this molecule has the potential to become an anticancer drug, in particular since it is selective for Ha-Ras-transformed malignant cells in comparison to parental non-malignant cells.

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