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# *IN VITRO* ANTI-PROLIFERATIVE ACTIVITY OF NOVEL HEXACOORDINATED TRIPHENYLTIN(IV) TRIFLUOROACETATE CONTAINING A BIDENTATE N-DONOR LIGAND

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The discovery of the antitumor activity of cisplatin led several research groups to investigate the possible therapeutic applications of other metal based compounds. In an attempt to develop novel metal based drugs with a different therapeutic profile to cisplatin, we have synthesized a new N,N-chelated organotin(IV) trifluoroacetate by the reaction of $Ph_3SnOCOCF_3$ with equimolar amounts of 2,9-dimethyl-1,10-phenanthroline (Neocuproine). The complex is characterized by FT-IR and multinuclear NMR ($^1H$, $^{13}C$, $^{19}F$ and $^{119}Sn$). FT-IR results authenticate the ligand coordination to the organotin moiety via nitrogen atoms. Furthermore, the cytotoxic activity of the free ligand (Neocuproine) and triorganotin(IV) complex towards human cervix carcinoma HeLa, human myelogenous leukemia K562 and normal immunocompetent cells, peripheral blood mononuclear cells PBMC is evaluated by the MTT (3-[4,5-dimetylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) method. The complex exhibits higher activities than antitumor drug cisplatin in all the tested cell lines. These results indicate that the studied triorganotin(IV) complex can be a potential anticancer agent for further stages of screening *in vitro* and/or *in vivo*.

**K e y w o r d s**: organotin(IV) complex, 2,9-dimethyl-1,10-phenanthroline, spectroscopic study, anticancer drug, cell line.

## INTRODUCTION

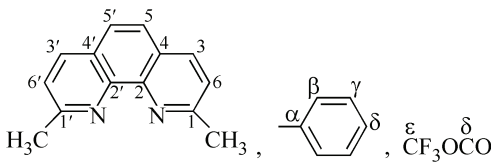
Many efforts have been paid to develop diagnostic and therapeutic pharmaceuticals of cancer or malignant tumor in nowadays. The widespread biological application of metal complexes in the treatment of numerous human diseases is a vigorously expanding area in biomedical and inorganic chemistry [ 1 ]. Cisplatin is the first drug from inorganic chemistry to have come under routine clinical use in medical oncology and its success placed the coordination chemists on the front line in the fight against cancer [ 2 ]. The discovery of cisplatin and related platinum complexes has stimulated the search for other active transition metal complexes with potential anticancer activity. Among the non-platinum metal compounds with antitumor activity, organotin complexes deserve particular notice. There is a broad spectrum of organotin compounds that can be prepared, with multiple mechanisms of action that may prevent or retard the development of drug resistance. Organotin(IV) compounds form an important series of compounds and have been widely used as biocides, as antifouling agents and for wood preservation. In recent years, investigations have been carried out to test their antitumor activity, and it has been observed that indeed several organotin compounds exert interesting antiproliferative activity

against a number of tumours or tumour cell lines, both *in vivo* and *in vitro* [ 3 ]. In general, triorganotin(IV) compounds display a higher biological activity than their di- and monoorganotin(IV) analogues, which has been related to their ability to bind to proteins [ 4 ]. There is a long-standing interest in chemistry and generally the structure of triorganotin(IV) esters of carboxylic acids because of the known catalytic and medicinal activity [ 5 ]. Upon coordination to a suitable metal centre, the biologically active carboxylic acids often become more effective and desirable drugs. Recent works also reveal higher antitumor activities for di- and triorganotin fluoro substituted carboxylates than their non-fluorinated analogs [ 6 ]. Furthermore, the stereochemistry of organotin compounds and the structural motif of the ligand are likely to play an important role within the biological activity of those materials. Aromatic, nitrogen-containing heterocyclic molecules are important ligands which form stable complexes with transition metal ions. 1,10-Phenanthroline (Phen) and substituted derivatives, both in the metal-free state and as ligands coordinated to transition metals, disturb the functioning of a wide variety of biological systems [ 7 ]. It is worth noting that the presence of phenanthroline rings and their methyl substitution was essential for the anticancer activity of both complexes because unsubstituted monochelate and bischelate complexes were less active [ 8 ]. As an extension of this research field, we are now interested in the development of the chemistry of a novel organotin complex obtained by the interaction of triorganotin(IV) trifluoroacetate with 2,9-dimethyl-1,10-phenanthroline. In order to compare its activity with that of cisplatin, the *in vitro* antitumor activity of the free ligand and the triorganotin complex has been tested against tumor cell lines human cervix carcinoma (HeLa), human myelogenous leukemia (K562) and normal immunocompetent cells, peripheral blood mononuclear cells (PBMC).

## EXPERIMENTAL

**Materials and physical measurements.** Commercial reagent grade 2,9-dimethyl-1,10-phenanthroline (2,9-$Me_2$phen, neocuproine), $Ph_3SnCl$, and $CF_3COOAg$ were used without further purification. Solvents were purified and dried according to the standard procedures. Melting points were determined in open capillaries and were uncorrected. FT-IR spectra were recorded on a Bomem MB-100 FT-IR spectrometer, using KBr pellets (400—4000 $cm^{-1}$). The $^1H$, $^{13}C$, $^{19}F$, $^{119}Sn$ NMR spectra were obtained by a Bruker AVANCE 500 spectrometer. The splitting of proton resonances in the reported $^1H$ NMR spectra are defined as s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet.

**Synthesis and characterization of [$(Ph)_3Sn(Cl)(OC(O)CF_3)(2,9\text{-}Me_2phen)$].** A solution containing $Ph_3SnCl$ (0.5 g, 1.29 mmol) dissolved in ethanol (20 ml) was reacted with an equimolar amount of $CF_3COOAg$ (0.28 g, 1.29 mmol) dissolved in the same solvent to give corresponding triorganotin(IV) trifluoroacetate. After stirring for 2 h at room temperature, the reaction mixture was filtered in order to eliminate finely suspended silver(I) halide. The filtered solution was treated with 2,9-$Me_2$phen (189 mg, 1.29 mmol) and stirred for 3 h and the solvent was evaporated, then the colorless crystals were separated out 5 days later. The numbering scheme of the ligand and organic groups is shown in Scheme 1. M.P. 162 °C. FT-IR ($cm^{-1}$): 1708 ν(C=O), 1601 ν(C=N), 1427—1501 ν(C=C), 455 ν(Sn—N), 548 ν(Sn—C). $^1H$ NMR(ppm): 2.9 (s, $CH_3$-phen, 6H), 7.75 (s, $H_{5,5'}$, 2H), 8.18, 8.17 (d, $H_{3,3'}$, 2H), 7.55, 7.53 (d, $H_{6,6'}$, 2H), 7.73—7.72 (m, $H_\beta$, Sn-$ph_3$), 7.51—7.48 (m, $H_{\gamma,\delta}$, Sn-ph). $^{13}C$ NMR(ppm): 26.2 ($CH_3$-phen), 145.5 (C-1), 137.7 (C-2), 136.7 (C-3), 127.2 (C-4), 125.8 (C-5), 124 (C-6), 136.6 (C-α), 130.9 (C-β), 129.8 (C-γ), 129.5 (C-δ), 160 (C-δ), 159 (C-ε), $^{119}Sn$ NMR(ppm): δ = –345.4, $^{19}F$ NMR: –75.15.




Scheme 1. Numbering scheme of the ligand and the organic group

**ANTIPROLIFERATIVE ASSAY *IN VITRO***

**Preparation of drug solutions.** Stock solutions of neocuproine and the studied tin complex were prepared in dimethyl sulfoxide (DMSO, Sigma Aldrich) at a concentration of 1000 μg/ml, sterilized by filtration through a 0.22 μm Millipore filter before use, and diluted by a cell culture medium to various working concentrations. The nutrient medium was RPMI-1640 (Gibco BRL, Scotland) supplemented with 10 % fetal bovine serum (FBS-Gibco BRL/Scotland). MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was dissolved (5 mg/ml) in phosphate buffer saline pH 7.2, and filtered through a 0.22 μm Millipore filter before use. HeLa cells were split using the 0.25—0.5 % TRED (Trypsin/EDTA-Gibco BRL, Scotland) medium prior to reaching 80 % confluence.

**Cell lines and cell culture.** The HeLa (human cervix carcinoma) cells (NCBI C115, National Cell Bank of Iran) and K562 (human myelogenous leukemia) cells (NCBI C122, National Cell Bank of Iran), were obtained from Pasteur Institute of Iran. The cells were cultured in the RPMI-1640 medium supplemented with 10 % heat inactivated fetal calf serum (FCS-Gibco BRL, Scotland) 2 mM L-Glutamine (Gibco BRL, Scotland ) and antibiotic-antimycotic, including streptomycin (100 μg/ml) and penicillin (100 IU/ml) (Sigma, USA) and Amphotecin B (2.5 μg/ml) incubated at 37 °C in a humidified 5 % $CO_2$ atmosphere. HeLa cells were cultured as a monolayer in the completed RPMI 1640 medium, while K562 cells were maintained as a suspension culture. The cells were grown at 37 °C in 5 % $CO_2$ and the humidified air atmosphere. Peripheral blood mononuclear cells (PBMC) were separated from whole heparinized blood from a healthy volunteer by density gradient centrifugation using lymphoprep (Nycomed, Oslo, Norway). Isolated cells, washed three times with PBS, then were counted and resuspended in the nutrient medium. Cells were counted by the improved Neubauer Hemocytometer slide and the Trypan blue dye exclusion method.

**Cytotoxicity assay.** HeLa cells were seeded (5000 cells per well) into 96-wells flat-bottom microtiter plates and incubated for 4 h prior to the addition of filtered 5 different concentrations of the studied compound. Final concentrations achieved in the treated wells were 0.001 μg/ml, 0.01 μg/ml, 0.1 μg/ml, 1 μg/ml, and 10 μg/ml. Each concentration was tested in quadruplicate on each cell line. The final concentrations (<0.1 %) of DMSO, were non-toxic to the cells. Only the complete medium was added to the cells in the control wells. The studied compound was added to the suspension of K562 cells (10000 cells per well) in 4 h after cell seeding, in the same final concentrations applied to the HeLa cells. Each assay included a blank containing the complete medium without cells. PBMC were seeded (200,000 cells per well) in the complete medium enriched with phytohemagglutinin (2.5 μg/ml, Sigma, USA) in 96 well microtiter plates and 4 h later the studied compounds were added to the wells, in quadruplicate, to 5 final concentrations, except to the control wells where the enriched cell culture medium only was added to the cells. The incubation time was 72 h; during the period the control cells showed exponential growth.

**Determination of target cell survival.** Cell survival was determined by the MTT test according to the method of Mosmann [ 9 ] and modified by Ohno and Abe [ 10 ]. Briefly, the cells were incubated for 72 h and then, 20 μl of an MTT solution (5 mg/ml) in phosphate buffer saline (1/10 of the total volume in a well) was added to the wells. Samples were incubated for further 4 h at 37 °C in the humidified atmosphere with 5 % $CO_2$ (Fig. 1).

Supernatants were removed and 100 μl of DMSO was added as a solvent to each well. The plate was shaken for 15 min by a shaker incubator in order to dissolve the formazan crystals. The optical density (OD) value was defined as the absorbance of each individual well, minus the blank value (blank is the mean optical density of the control cells). Finally, the absorbance at 570 nm (test wavelength) and with a reference filter of 630 nm was measured using an ELISA microplate reader (Stat Fax-2100, USA). All experiments were performed three times and the percentage of cytotoxicity was calculated according to following formula:

$$\%\text{Cytotoxicity} = 1 - \frac{\text{mean absorbance of toxicant treated cells}}{\text{mean absorbance of negative control}} \times 100$$

$$\%\text{Viability} = 100\ \% - \%\text{Cytotoxicity}$$

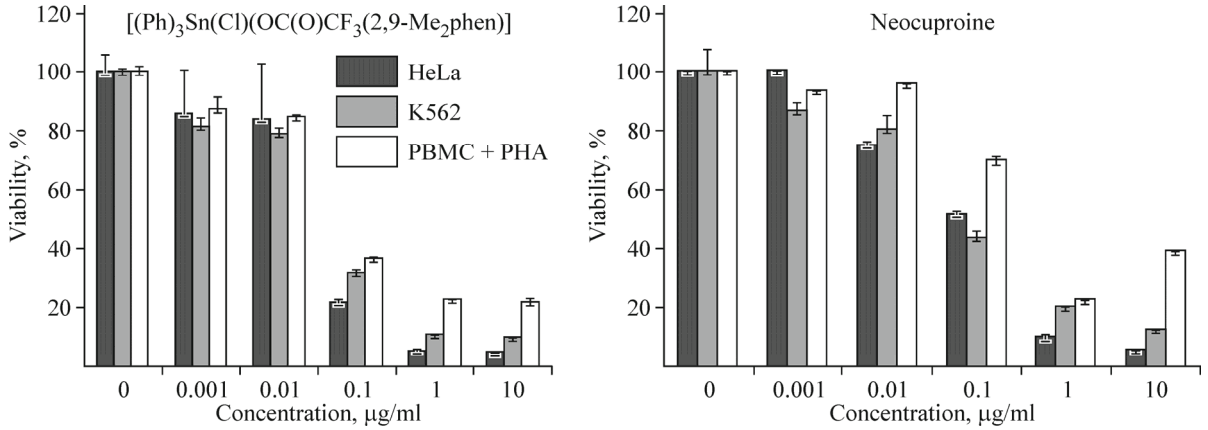


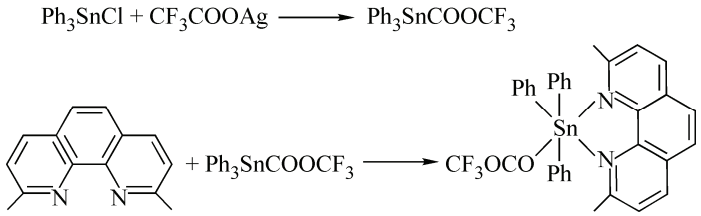

*Fig. 1.* Cytotoxicity graphs from typical MTT assays showing the effect of the studied compounds on the viability of K562, HeLa, and PBMC+PHA (PBMC stimulated with PHA) cells

## STATISTICAL ANALYSIS

After subtracting the solvent toxicity, the concentration giving 50 % inhibition ($IC_{50}$) was determined for the test samples by the nonlinear regression analysis of the curves. Graph Pad Prism version 4.00 was used to calculate $IC_{50}$. The mean difference among the groups was calculated by a paired Student′s t-test, one-way and repeated measures ANOVA ($p < 0.05$).

## RESULTS

**Synthetic aspects.** N,N-chelated triorganotin(IV) trifluroacetate [$(Ph)_3Sn(Cl)(OC(O)CF_3)$(2,9-$Me_2$phen)] was prepared, in a very high yield by the reaction of $Ph_3SnOCOCF_3$ with 2,9-dimethyl-1,10-phenanthroline as shown in Scheme 2.

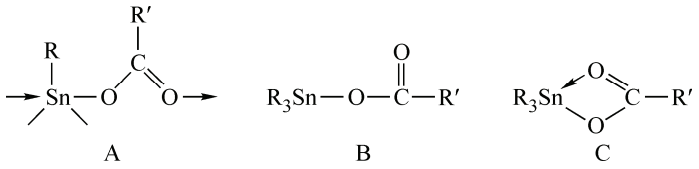



Scheme 2. Synthesis of the [$(Ph)_3Sn(Cl)(OC(O)CF_3)$(2,9-$Me_2$phen)] complex

**Spectroscopic studies.** The IR data are consistent with the formation of well-defined compounds with the compositions. The ν(C=N) band at 1672 $cm^{-1}$ found in the spectrum of the free ligand is shifted to a lower frequency of 1601 $cm^{-1}$ in the complex, showing that the nitrogen atom of the ligand coordinates with the central tin atom. For organotin(IV) carboxylates, IR spectroscopy is helpful, pertaining to the coordination mode of the COO moiety. The carboxylate group is able to coordinate to metal ions by different coordination modes based on the IR asymmetric and symmetric stretching of the COO group and the stretching for Sn—C and Sn—O (Scheme 3). It is generally believed that different values in Δν between the asymmetric ($\nu_{as}$(COO)) and symmetric ($\nu_{sym}$(COO)) absorption frequencies can distinguish the ligating mode of a carboxylate moiety, that is, the value smaller than 200 $cm^{-1}$ indicates that the carboxylate moiety is bidentate, while the value larger than 200 $cm^{-1}$ indicates that the carboxylate moiety is unidentate [ 11 ].

In the infrared spectra of [$(Ph)_3Sn(Cl)(OC(O)CF_3)$(2,9-$Me_2$phen)] the $\nu_{as}$(COO) and $\nu_{sym}$(COO) bands appear at 1708 $cm^{-1}$ and 1479 $cm^{-1}$ respectively. The difference between these frequencies $\Delta[\nu_{as}(COO) - \nu_{sym}(COO)]$ is close to that found for monodentate carboxylate groups (229 $cm^{-1}$). Further, the bonding of 2,9-dimethyl-1,10-phenanthroline to tin is supported by the appearance of a new

band near 455 $cm^{-1}$ in the compound, which may be assigned to (Sn—N). Compared with the free ligand the peak appeared at 548 $cm^{-1}$ is assigned to Sn—C, which confirmed the existence of Sn—C bonds for the complex.




Scheme 3. Possible structural motifs for triorganotin(IV) esters of carboxylic acids

The $^{1}H$ NMR spectra further support the bonding pattern as discussed above. The $^{1}H$ NMR spectral data of the corresponding triorganotin(IV) complex was recorded in DMSO. The $^{1}H$ NMR spectra of the complex exhibit a sharp singlet at 2.9 ppm corresponding to the protons of methyl groups attached to the phenantroline ring in the aliphatic region, while the aromatic part of the ligand gave two doublets at 8.18—8.17 ppm and 7.55—7.53 ppm and one singlet at 7.75 ppm due to three non-equivalent sets of protons. The signals for the protons of the phenyl groups attached to Sn were distinguished in two sets. The ortho protons were observed at down field (7.73—7.72 ppm) and these for meta and para protons at upfield (7.51—7.48 ppm). The $^{13}C$ and $^{19}F$ NMR spectra of the complex, were recorded in order to elucidate the presence of the $CF_3COO$ substituent. The presence of the exact number of carbon resonances for the ligand and phenyl groups, as anticipated from the structures, in the spectra of the studied complex validate the formation of the complex. A typical signal at low field (160 ppm) was observed in the spectra and is assigned to the carbon atom of the carboxylate group. In addition, the methyl groups bonded to the phenantroline ring were observed in the spectrum as one signal at 26.2 ppm. The signal of the $CF_3$ group in the $^{19}F$ NMR spectra was found at –75.15 ppm. The values of the $^{119}Sn$ chemical shift for organotin(IV) compounds may be used to give tentative indications of the environment around tin atoms. In the range of +200 to –60 ppm, –90 to –190 ppm, –210 to –400 ppm, –440 to –540 ppm, tin coordination numbers are 4, 5, 6, and 7 respectively [ 12 ]. The triorganotin complex under investigation exhibits a single $^{119}Sn$ chemical shift at –345.4 ppm, indicating that the tin atom is six-coordinated in the studied complex.

**Cytotoxic studies.** The cytotoxicity of neocuproine, $[(Ph)_3Sn(Cl)(OC(O)CF_3)(2,9\text{-}Me_2phen)$, and $[Pt(NH_3)_2Cl_2]$ as a reference drug against human cervix carcinoma (HeLa), human myelogenous leukemia (K562), and on normal immunocompetent cells, peripheral blood mononuclear cells (PBMC) was determined with the aid of the MTT assay, which serves as an index of cell viability by measuring the reduction of tetrazolium salt to purple formazan by the mitochondrial enzyme activity of succinate dehydrogenase in living cells. Cells were exposed to various concentrations of each compounds for 72 h. Results show that the cytotoxicity of the free ligand and $[(Ph)_3Sn(Cl)(OC(O)CF_3(2,9\text{-}Me_2phen)$ is dose-dependent (Fig. 1). The anticancer activity of the free ligand against the following cancer cell lines K562, HeLa and PBMCs are 3, 17, and 73 times more potent than cisplatin respectively, but less cytotoxic compared to the triorganotin complex.

The studied organotin(IV) complex exhibited strong *in vitro* antitumor activities toward human cell lines, which are even higher than those of cisplatin, the widely clinically used drug. The investigated complex has a similar level of antiproliferative activity (0.04 μg/ml) on each tumour cell line and on stimulated PBMC (Table 1). This similarity in cell viability indicates that the activity of the complex may not be cell-type specific. The triorganotin compound exhibited almost the same activity on normal immunocompetent cells PBMC.

## CONCLUSIONS

In summary, 2,9-dimethyl-1,10-phenanthroline reacts with triphenyltin(IV) trifluoroacetate in the 1:1 molar ratio in methanol, resulting in a complex with the stoichiometry [(Ph)3Sn(Cl)(OC(O)CF3)··(2,9-Me2phen)]. The FT-IR, $^{1}H$, $^{13}C$ and $^{119}Sn$ NMR data on the complex revealed that the ligand

Table 1

$IC_{50}$ (μg/mL) *for* 72 h *of action of the studied compounds and cisplatin on* HeLa, K562 *and PBMC stimulated with PHA determined by the MTT test*

| Compound | HeLa | K562 | PBMC+PHA |
|---|---|---|---|
| Neocuproine | 0.076 | 0.58 | 0.107 |
| $[(Ph)_3Sn(Cl)(OC(O)CF_3(2,9\text{-}Me_2phen)$ | 0.04 | 0.04 | 0.04 |
| $[Pt(NH_3)_2Cl_2]$[a] | 1.32 | 1.71 | 7.8 |

[a] Standard drug, *cis*-$[Pt(NH_3)_2(Cl)_2$.

binds the metal as a bidentate [N,N] chelating agent such that the metal adopts a hexagonal geometry. The *in vitro* cytotoxic activity has been evaluated against the tumor cell lines human cervix carcinoma (HeLa), human myelogenous leukemia (K562) and normal immunocompetent cells, peripheral blood mononuclear cells (PBMC). The $IC_{50}$ results reported in Table 1 reveal that the tin complex with the neocuproine ligand is much more active than the parent ligand against the evaluated cell lines. Furthermore, the antitumor activity data demonstrate that the investigated organotin(IV) complex has a significant antitumor effect against the tested cell lines, observing much lower $IC_{50}$ values than cisplatin. Following on from these results, intensive studies on the mechanism of action of a novel synthesized complex against the different studied cancer cells will be carried out.

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