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Original article

Biological studies of new organotin(IV) complexes of thioamide ligands

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Abstract

New organotin(IV) complexes with heterocyclic thioamides 2-mercapto-benzothiazole (Hmbzt), 5-chloro-2-mercapto-benzothiazole (Hcmbzt) and 2-mercapto-benzoxazole (Hmbzo) of formulae $[(C_6H_5)_3Sn(mbzt)]$ (**1**), $[(C_6H_5)_3Sn(cmbzt)]$ (**3**) and $[(C_6H_5)_2Sn(cmbzt)_2]$ (**4**), together with the already known $[(C_6H_5)_3Sn(mbzo)]$ (**2**), $[(n\text{-}C_4H_9)_2Sn(cmbzt)_2]$ (**5**) and $[(CH_3)_2Sn(cmbzt)_2]$ (**6**) were used to study their influence on the peroxidation of oleic acid. The influence of complexes (**3**)–(**6**) upon peroxidation of oleic acid showed that the formation of reactive radicals caused the initiation of the chain radical oxidation of the substrate. The influence of complexes (**1**)–(**6**) upon the catalytic peroxidation of linoleic acid by the enzyme lipoxygenase (LOX) was also studied and compared to those of cisplatin. Compounds (**1**)–(**6**) were finally tested for *in vitro* cytotoxicity against leiomyosarcoma cells.

Keywords: Bioinorganic chemistry; Organotin(IV) complexes; S ligands–heterocyclic thioamides; Catalytic peroxidation of fatty acids; Cytotoxic action

1. Introduction

The analogous to carboplatin [1–4] organotin(IV) complexes are known to possess anti-tumor properties [2,5]. In 1985 and later Gielen et al. published a series of papers on the subject [2] even though the anti-tumor properties of tin complexes were already known [2]. Since then, more research groups have worked in the field [6]. Despite this, the mechanism of anti-tumor action of organotin compounds is still unknown. On the other hand, it is well known that many drugs which inhibit the growth of tumor cells act either by interfering with the bases and/or nucleotides of the double helix of

Abbreviations: ID_{50}, 50% inhibitory dose; MCF-7, mammary cancer; WiDr, colon carcinoma; DNA, deoxyribonucleic acid; 5′-CMP, cytidine-5′-monophosphate; 5′-dCMP, 2′-deoxycytidine-5′-monophosphate; 5′-UMP, uridine-5′-monophosphate; 5′-IMP, inosine-5′-monophosphate; 5-GMP, guanosine-5′-monophosphate; ATPase, adenosine 5′-triphosphatase.

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DNA or with the metalloenzymes that are necessary for the rapid growth of malignant cells [7,8].

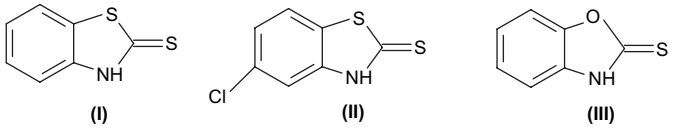
For the mechanism of action of organotin(IV) compounds, multinuclear 1D and 2D NMR studies [9] of the interaction between diethyltin(IV) moiety and 5′-CMP, 5′-dCMP, 5′-UMP as well as 5′-IMP and 5-GMP in aqueous solutions have shown that at low pH values (<4) organotin(IV) moiety is involved in bonding with the phosphate group of the nucleotides studied. At pH > 9.5, the Et_2Sn(IV) moiety reacts with the O(2′) and O(3′) oxygen atoms of the sugar unit of the nucleotide, while at intermediate pH values (4.0–9.5), no evidence for the Et_2Sn(IV)–nucleotide interaction was found [9]. These results may indicate that organotin's anti-cancer activity may not involve DNA at physiological conditions.

On the other hand, organotins were proposed to interact with the high-affinity site of ATPase (histidine only) and the low-affinity site of ATPase and haemoglobins (histidine and cysteine) [10]. These results prompted us to investigate the correlation between enzyme inhibitions by organotins and their anti-tumor activity. Lipoxygenase (LOX) is an enzyme that takes part in the metabolism of arachidonic acid. LOX catalyzes the oxidation of arachidonic acid to leukotrienes, in an essential mechanism for the cell life [11a,11b]. Prostaglandins the final products formed from the metabolism of arachidonic acid contributed to tumorigenesis acting as angiogenesis factors [12a]. Linoleic acid on the other hand, discovered in beef and dairy products, was proven to be a potential mutagen inhibitor [12b].

Lipophilic organotin compounds R_nSnX_{4-n} are membrane active xenobiotics and when accumulating in the lipid bilayer of cellular membranes might induce membrane associated oxidative stress in living organism [13a–13c]. The principal route of these processes is associated with the promoting effect of lipid peroxidation in the presence of organotins [14a,14b]. The organotin compounds R_nSnX_{4-n} are capable of interacting with peroxyl radicals $R'OO^{\bullet}$ formed in the peroxidation of unsaturated fatty acids R′H by dioxygen and produce active organic radicals $R^{\bullet}$ due to the homolytic cleavage of Sn–C bonds in radical substitution reactions [14c].

In this study, the correlation of LOX inhibitory activity and the anti-tumor activity of complexes $[(C_6H_5)_3Sn(mbzt)]$ (**1**), $[(C_6H_5)_3Sn(cmbzt)]$ (**3**), and $[(C_6H_5)_2Sn(cmbzt)_2]$ (**4**) {where Hmbzt is 2-mercapto-benzoxazole (Scheme 1(**I**)) and Hcmbzt is 5-chloro-2-mercapto-benzothiazole (Scheme 1(**II**))} [15] and $[(C_6H_5)_3Sn(mbzo)]$ (**2**) [16a] (Hmbzo = 2-mercapto-benzoxazole (Scheme 1(**III**)), $[(n\text{-}C_4H_9)_2Sn(cmbzt)_2]$ (**5**) [16b] and $[(CH_3)_2Sn(cmbzt)_2]$ (**6**) [6a] are reported. Such an inhibition may be the cause of the anti-tumor action of tin anti-tumor complexes.

More particularly (**1**)–(**6**) were tested for *in vitro* cytotoxicity against leiomyosarcoma cells from the Wistar rat [17].



Scheme 1.

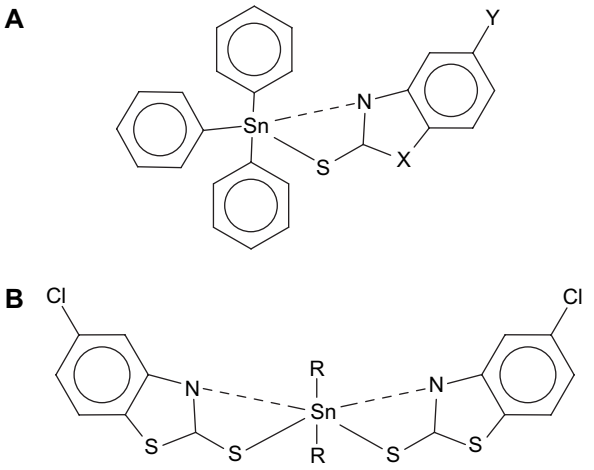


Fig. 1. Formulae of the complexes (**1**)–(**6**). $[(C_6H_5)_3Sn(mbzt)]$ (**1**) (A; X = S, Y = H), $[(C_6H_5)_3Sn(mbzo)]$ (**2**) (A; X = O, Y = H), $[(C_6H_5)_3Sn(cmbzt)]$ (**3**) (A; X = S, Y = Cl), $[(C_6H_5)_2Sn(cmbzt)_2]$ (**4**) (B; R = C_6H_5–), $[(n\text{-}C_4H_9)_2Sn(cmbzt)_2]$ (**5**) (B; R = *n*-C_4H_9–) and $[(CH_3)_2Sn(cmbzt)_2]$ (**6**) (B; R = CH_3–).

Their anti-tumor activity was studied in relation to the mechanism of inhibition activity of the complexes towards the catalytic oxidation of linoleic acid to hydroperoxylinoleic acid by the enzyme lipoxygenase [11b,12a]. An interesting correlation between anti-tumor action and LOX inhibition of the complexes studied was observed.

2. Chemistry

2.1. Synthesis and structures

Details of the synthesis of compounds (**1**), (**2**), (**5**) and (**6**) have been reported elsewhere [6a,15,16]. Compounds (**3**) and (**4**) have been prepared by direct reaction of diphenyltin oxide $(C_6H_5)_2SnO$ or triphenyltin hydroxide with 5-chloro-2-mercapto-benzothiazole [15].

The structures of the complexes used are included in Fig. 1.

The structures of compounds (**1**)–(**3**) consist of a de-protonated thioamide ligand bonded to $[Ph_3Sn(IV)]$ moiety through sulfur atom. A weak Sn–N interaction completes the coordination sphere around the metal center. The geometry around Sn atom is basically tetrahedral. Tin is coordinated with, two carbons of phenyl groups and two sulfur atoms. The weak N···Sn interaction in plane may be interpreted as a slight shift towards a trigonal bipyramidal structure. In (**4**)–(**6**), the distortion leads to an octahedral arrangement around tin, taking into account the two weak N···S interactions.

3. Results and discussion

3.1. The influence of organotin(IV) complexes (3)–(6) upon the peroxidation of oleic acid

Fig. 2 shows the effect of organotin complexes (**4**)–(**6**) on the accumulation level of oleic acid hydroperoxides at 65 and 37 °C, respectively.

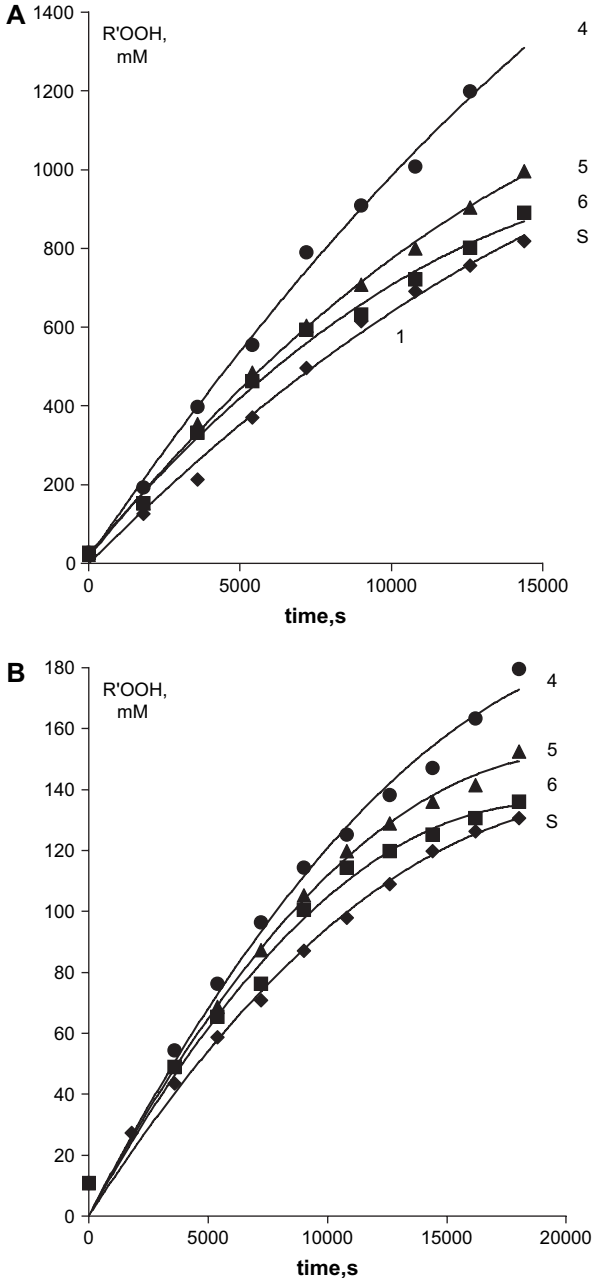


Fig. 2. Kinetic curves of R′OOH formation in the presence of 1 mmol/l additives at 65 °C (A) and 37 °C (B) oleic acid (S) without additives; and in the presence of complexes Me_2SnL_2 (**6**), $(n\text{-}Bu)_2SnL_2$ (**5**) and Ph_2SnL_2 (**4**).

The effect of all organotin complexes R_2SnL_2 is manifested by the increase of the hydroperoxide content in the initial period (Fig. 2A,B; curves 4–6). The data presented show the influence of the organic group nature upon the effectiveness of R_2SnL_2. The initial rate constants k of R′OOH accumulation at 65 °C are 3.83 ± 0.03; 4.04 ± 0.03; 4.33 ± 0.01 and $4.32 \pm 0.02 \times 10^{-4}\ s^{-1}$ for pure oleic acid (S) and Me_2SnL_2 (**6**), $(n\text{-}Bu)_2SnL_2$ (**5**) and Ph_2SnL_2 (**4**), respectively. The analogous values at 37 °C are lower because of the decrease of the substrate peroxidation rate at physiological temperature and are 2.51 ± 0.04; 2.56 ± 0.03; 2.66 ± 0.03 and $2.7 \pm 0.03 \times 10^{-4}\ s^{-1}$ for pure oleic acid (S) and Me_2SnL_2 (**6**), $(n\text{-}Bu)_2SnL_2$ (**5**) and Ph_2SnL_2 (**4**), respectively.

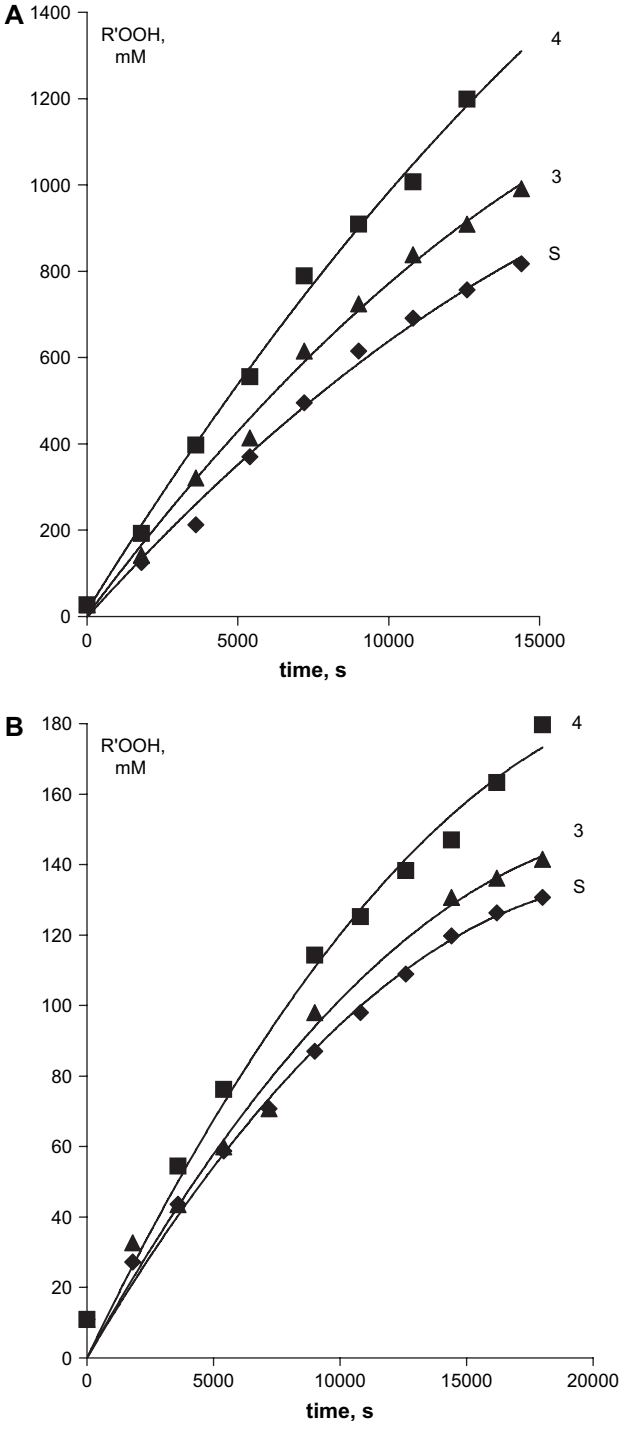


Fig. 3. Kinetic curves of R′OOH formation in the presence of 1 mmol/l additives at 65 °C (A) and 37 °C (B) oleic acid (S) without additives; Ph_3SnL (**3**) and Ph_2SnL_2 (**4**).

The number of R groups in R_nSnL_m influences the effectiveness of the complexes as promoters as well, as it has been observed for the pair of phenyl derivatives (Fig. 3). At both temperatures diphenyltin complex Ph_2SnL_2 (**4**) is more active in the initiation of peroxidation process than the triphenyltin analogue Ph_3SnL (**3**) ($k = 4.32 \pm 0.02$; 4.06 ± 0.01 and 2.7 ± 0.03; $2.410.03 \times 10^{-4}\ s^{-1}$ at 65 °C and 37 °C for Ph_2SnL_2 (**4**) and Ph_3SnL (**5**), respectively).

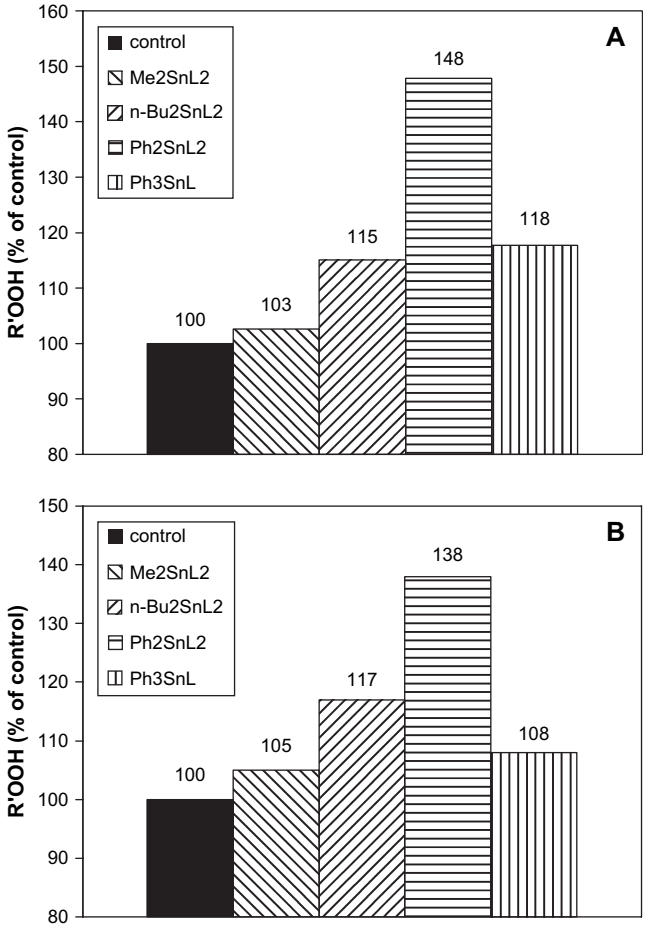


Fig. 4. The relative content of R′OOH in oleic acid without additives and in the presence of 1 mmol/l oleic acid (S) without additives; and in the presence of complexes Me_2SnL_2 (**6**), $(n\text{-}Bu)_2SnL_2$ (**5**), Ph_2SnL_2 (**4**) and Ph_3SnL at 65 °C (A) and 37 °C (B) after 2.5 h and 5 h, respectively.

The comparative data for the R′OOH total content in pure oleic acid and in the presence of R_nSnL_m after 2.5 h at 65 °C and after 5 h at 37 °C are given in Fig. 4. A significant increase of R′OOH at various temperatures has been observed, as compared to the oleic acid auto-oxidation.

The relative values of the total content of hydroperoxides in the presence of organotin complexes follow the same order of the organic group influence, as it was presented in kinetic measurements (Figs. 2 and 3).

The bonding of organotin moieties to the S-atom makes the complexes inert towards the free HS-groups in proteins as it has been mentioned above. However, the complexes possessing σ-bonded organic groups R are reactive towards the peroxyl radicals $R'OO^{\bullet}$ formed in oleic acid media.

The formation of reactive radicals $R^{\bullet}$ and/or $[R_nSnL_{4-n}]^{\bullet}$ on the other hand, might be the reason causing the initiation of the chain radical oxidation of the substrate [18a]. The half-life period of the radical formed might influence the promoting effect. As it is presented in Fig. 2 the more stable the organic C-centered radicals $R^{\bullet}$ formed in the oxidative medium ($CH_3^{\bullet} < CH_3CH_2CH_2CH_2^{\bullet} < C_6H_5^{\bullet}$) [18b], the more effective promoters of the peroxidation the corresponding complexes appear.

In order to examine further the above hypothesis we studied the EPR spectra of the Ph_3SnCl, Me_2SnCl_2, Hcmbzt and their complexes $Ph_3Sn(cmbzt)$ (**3**) and $Me_2Sn(cmbzt)_2$, (**6**). Fig. 5 shows frozen solution Electron Paramagnetic Resonance (EPR) spectra of the Me_2SnCl_2 (a), Ph_3SnCl (b), Hcmbzt (c) and their complexes $Ph_3Sn(cmbzt)$ (**3**) (d) and $Me_2Sn(cmbzt)_2$, (**6**) (e) after UV irradiation. Irradiation was performed with a 1000 W Oriel [Hg(Xe)] ozone-free lamp in liquid nitrogen, 77 K. The spectra shown correspond to 20 s irradiation. Before UV irradiation no EPR spectrum was detectable for any of the samples. Irradiation with visible light >400 nm did not induce any EPR signal.

The hyperfine splitting in the EPR spectrum of Me_2SnCl_2 (Fig. 5a) is due to the CH_3 protons. No such spectrum is detected in $Me_2Sn(cmbzt)_2$, (**6**) (Fig. 5d) indicating that the spin density is not delocalized onto the methyl groups. The spectrum of Hcmbzt (Fig. 5c) is seen in the spectra $Ph_3Sn(cmbzt)$ (**3**) (Fig. 5e) and $Me_2Sn(cmbzt)_2$, (**6**) (Fig. 5d). This indicates that the spin density in complexes $Ph_3Sn(cmbzt)$ (**3**) and $Me_2Sn(cmbzt)_2$, (**6**) is localized to a large degree on the Hcmbzt ligand.

3.2. Study of the peroxidation of linoleic acid by the enzyme lipoxygenase in the presence of complexes (1)–(6)

The influence of complexes (**1**)–(**6**) on the oxidation of the linoleic acid by the enzyme LOX was studied in a wide concentration range. The degree of LOX activity (A, %) in the presence of the complexes was calculated according to the method described previously [19a]. Fig. 6 compares the inhibitory effect of complexes (**1**)–(**6**) in various concentrations. It is shown that the catalytic activity of LOX decreased significantly in the presence of low concentrations (about 5–50 μM) of these complexes in contrast to cisplatin which shows no such activity. Among tri-organotin(IV) complexes (**1**)–(**3**), complex (**3**) exhibits the strongest inhibitory activity (IC_{50} values found: 19 μM (**1**), 21 μM (**2**), 16 μM (**3**), respectively, Fig. 6A) while among the di-organotin(IV) complexes (**4**)–(**6**), (**4**) is the stronger inhibitor towards LOX (IC_{50} values found: 10 μM (**4**), 13 μM (**5**), 14 μM (**6**), respectively, Fig. 6B) [19a]. Fig. 6C compares the inhibitory activity between (**3**) and (**4**). As it is shown (**4**) inhibits the LOX activity stronger. Thus the inhibitory activity of complexes (**1**)–(**6**) follow the order (**4**) > (**5**) = (**6**) > cisplatin, (**3**) > (**1**) > (**2**) > cisplatin. Surprisingly, the inhibitory activity of complexes tested towards the catalytic oxidation of linoleic acid follow the same order of promotion caused by these complexes in the oxidation of oleic acid non-enzymatically.

In order to evaluate the inhibition type, the enzymatic reaction was studied by steady-state kinetics at various substrate concentrations (varied from 0.01 to 0.1 mM) in the absence and presence of the complexes (15 μM). The experimental data were processed by a graphical method in Lineweaver–Burk coordinates (double reciprocal method). The kinetic parameters were estimated from the slope and intercept of the line. The K_m of the enzyme is 0.035 mM with V_{max} of

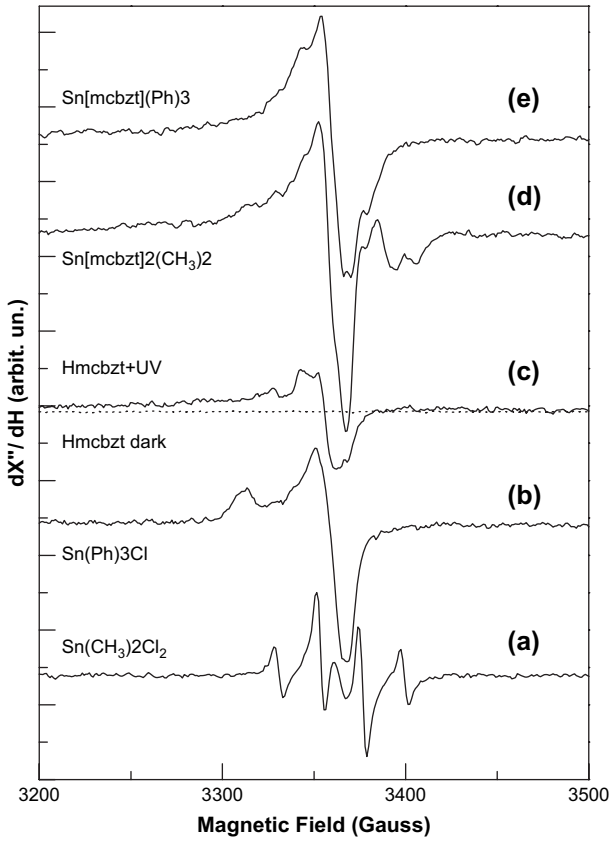


Fig. 5. Electron Paramagnetic Resonance (EPR) spectra of the Me_2SnCl_2 (a), Ph_3SnCl (b), Hcmbzt (c) and their complexes Ph_3Sn(cmbzt) (**3**) (d) and $Me_2Sn(cmbzt)_2$, (**6**) (e) after UV irradiation.

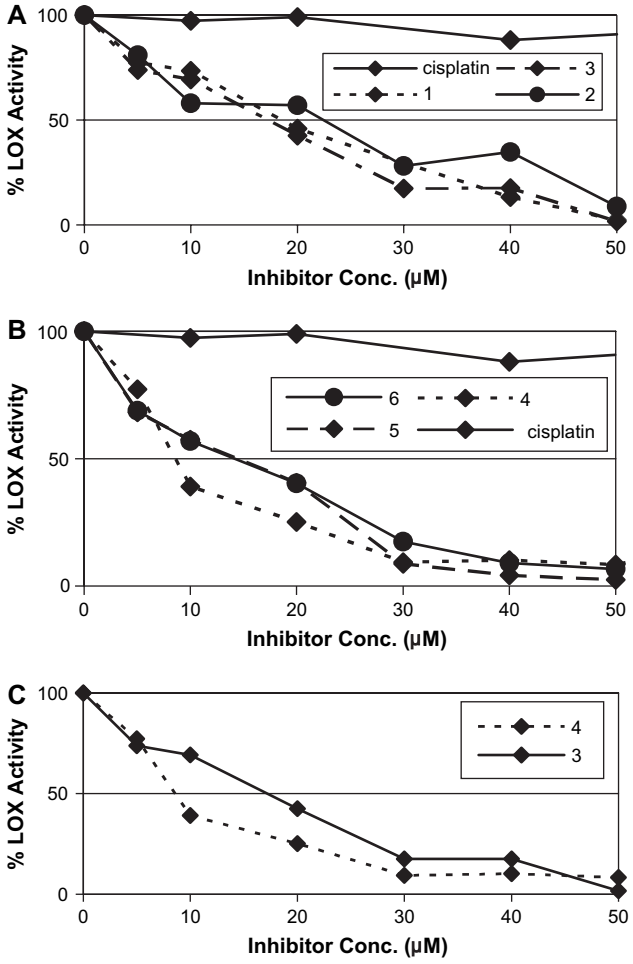


Fig. 6. % LOX activity on the oxidation of linoleic acid in the presence of inhibitors (**1**)–(**6**). (A): % LOX activity in the presence of triphenyltin(IV) complexes (**1**)–(**3**) in comparison with the corresponding activity with cisplatin. (B): % LOX activity of di-organotin(IV) complexes (**4**)–(**6**) in comparison with the corresponding activity with cisplatin. (C): comparison between the complexes with strongest inhibitory activity from the tri- and di-organotin complex groups.

27.5 mM/s [19a], while the corresponding apparent values for complexes (**1**)–(**6**) are found to be higher (0.070 mM (**1**), 0.047 mM (**2**), 0.051 mM (**3**), 0.037 mM (**4**), 0.038 mM (**5**) and 0.048 mM (**6**), respectively) with V_{max} values to be lower than the corresponding ones of the enzyme (20.7 mM/s (**1**), 25.4 mM/s (**2**), 19.7 mM/s (**3**), 20.0 mM/s (**4**), 18.0 mM/s (**5**) and 22.6 mM/s (**6**), respectively). Thus, the compounds studied inhibit the enzyme with a mixed inhibition mechanism [19b]. In this mechanism both the EI (enzyme–inhibitor) and ESI (enzyme–substrate–inhibitor) complexes are formed [19b]. This occurs when the inhibitor binds at a site away from the substrate binding site, causing a reduction in the catalytic rate which may lead to cell death. Such inhibitors could not act as anti-inflammatory agents.

3.3. Computational methods – docking study

The high inhibitory effect of compounds (**1**)–(**6**) towards LOX activities prompted us to perform molecular docking studies to understand the complex–protein interactions. Table 1 shows the binding energy (E) when the substrate (S = linoleic acid) binds in the enzyme LOX (E) for the formation of ES complex ($E = -7.89$ kcal/mol) [19a] and the corresponding binding energies when the inhibitors (I), bind for the formation of either ESI or EI complexes. According to the E values of ES in contrast to those of EI and ESI indicate that both ESI and EI complexes could be formed in agreement with the experimental findings for the mechanism of LOX inhibition presented above. Fig. 7 shows the area of the binding sites of the inhibitors studied here. The volume binding site is found to be 916.74 SA (Richards' surface) [20a] or 3027.57 MS [20b], (Connolly's surface). Thus, according to computational studies, inhibitor binds to a site away from the substrate binding site while both ESI and EI complexes could be formed. These findings further support the reversible mixed inhibition mechanism which were also found from kinetic studies.

3.4. Biological tests

Compounds (**1**)–(**6**) were tested for cytotoxic activity against leiomyosarcoma cells from the Wistar rat. Leiomyosarcoma cells (mesenchymal tissue) survival (%) at 24 h under variable concentration (μM) of (**1**)–(**6**) are shown in Fig. 8.

Table 1
Binding energy (E) values when the substrate (S = linoleic acid) binds to the enzyme LOX (E) with or without the presence of the inhibitor (I)

Substrate or inhibitor	Energy of ES or ESI (kcal/mol)	Energy of EI (kcal/mol)	d(Sn–Fe[a]) in ESI (Å)	d(Sn–C[b]) in ESI (Å)	Amino acid residues form the docking cavity of the substrate and/or the inhibitor	Ref
Linoleic acid	−7.89	–	–	–	76Ala, 533Arg, 760Asp, 761Glu, 249Leu, 110Lys, 15Met, 108Phe, 759Ser, 762Val	[19a]
(**1**)	−8.6	−9.2	23.0	7.3	254Ala, 242Arg, 18Asn, 245Asn, 247Gly, 248His, 257Ile, 249Leu, 17Lys,15Met, 108Phe, 16Pro, 130Trp	[c]
(**2**)	−9.5	−9.6	23.0	4.9	254Ala, 242Arg, 18Asn, 245Asn, 247Gly, 248His, 257Ile, 249Leu, 17Lys,15Met, 108Phe, 16Pro, 130Trp	[c]
(**3**)	−9.0	−11.2	22.8	6.8	255Ala, 18Asn, 247Gly, 248His, 257Ile, 249Leu, 17Lys, 250Lys, 15Met, 108Phe, 16Pro, 130Trp, 251Ser	[c]
(**4**)	−9.1	−9.5	25.1	10.5	76Ala, 18Asn, 253Asp, 78Glu, 75Gly, 77Gly, 259Leu, 250Lys, 252Lys, 251Ser, 759Ser	[c]
(**5**)	−8.5	−9.1	23.9	9.6	254Ala, 242Arg, 18Asn, 247Gly, 248His, 249Leu, 17Lys, 15Met, 108Phe, 16Pro, 251Ser, 130Trp, 105Val	[c]
(**6**)	−8.4	−8.3	23.9	9.6	254Ala, 18Asn, 256Glu, 247Gly, 248His, 257Ile, 249Leu, 17Lys, 15Met, 108Phe, 16Pro, 130Trp	[c]

[a] Iron of the active site of LOX.
[b] C(8) of linoleic.
[c] Present work.

Six replications per treatment group for the cytotoxic experiments were used. These results show the anti-proliferate effects for all complexes.

Among tri-organotin complexes (**1**)–(**3**) complex (**3**) shows the higher cytotoxic activity (Fig. 8A), while among di-organotin (**4**)–(**6**) derivatives complex (**4**) exhibits higher cytotoxic activity (Fig. 8B). Between tri- and di-organotin complexes, both (**4**) and (**3**) are found to exhibit almost the same strong cytotoxic activity (Fig. 8C).

4. Conclusions

It is well known that many drugs with anti-proliferative activity act either by interfering with the bases and/or nucleotides of the double helix of DNA or with the metalloenzymes that are necessary for the rapid growth of malignant cells [7,8]. However, it is proved that diethyltin(IV) moiety reacts with nucleobases only at low and high pH values but not at neutral pH [9]. Organotin(IV) complexes (**1**)–(**6**) are found to inhibit strongly the peroxidation of linoleic acid by the enzyme lipoxygenase with the same rate that these compounds are found to promote the peroxidation of oleic acid non-enzymatically. The free radical mechanism proposed for the promotion of fatty acids peroxidation [18a] is further supported by EPR studies, reported here. However, in our case the spin electron is located onto the ligand of the complexes and not on the alkyl or aryl groups derived by the R–Sn bond cleavage [14a]. On the other hand, according to Huber and Saxena [6g] the structures of all organotins anti-tumor active compounds are characterized by (i) the availability of coordination positions at Sn and (ii) the occurrence of relatively stable ligand–Sn bonds, e.g. Sn–N and Sn–S and their slow hydrolytic decomposition. In case of complexes (**1**)–(**6**) the highest cytotoxic activity is shown by the six coordinated complex (**4**) which has no free coordination position while its Sn–S and Sn–N bond distances are found to be shorter than those found in tri-organotin complexes [6g]. Anti-proliferative activity of the complexes studied here, follows the same rate found for the promotion of oleic acid peroxidation, non-enzymatically as well as the LOX activity inhibition. Thus, among tri-organotin complexes (**1**)–(**3**) complex (**3**) shows the higher cytotoxic activity, while among di-organotin (**4**)–(**6**) derivatives, complex (**4**) shows the higher cytotoxic activity. Between tri- and di-organotin complexes, both (**4**) and (**3**) are found to exhibit almost the same strong cytotoxic activity which is stronger than that of the corresponding activity of cisplatin. Therefore, a relation between the non-enzymatic peroxidation of fatty acids and the LOX activity inhibition is found which may also be related with the cytotoxic activity of organotin complexes. The geometrical feature of this type of complexes seems to play a less important role. In this case the free radicals formed from the reaction between organotin complexes and the peroxyl radicals, which are responsible for the promotion of the peroxidation of fatty acids, may also explain the LOX inhibition caused by complexes (**1**)–(**6**) with a mixed type mechanism. Since the spin electron of the radicals is located on

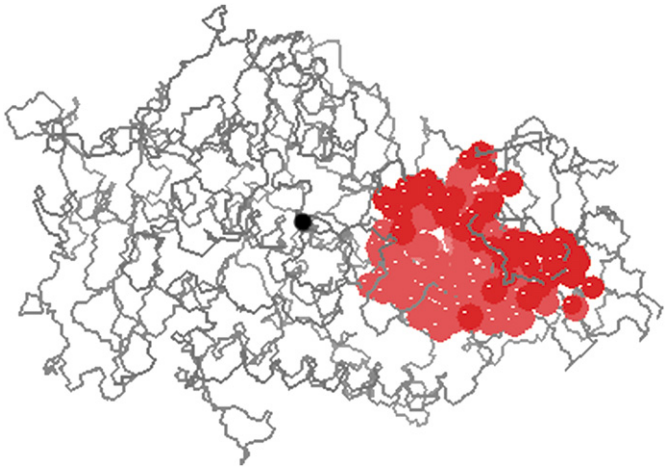


Fig. 7. Binding sites of the inhibitors (**1**)–(**6**) studied.

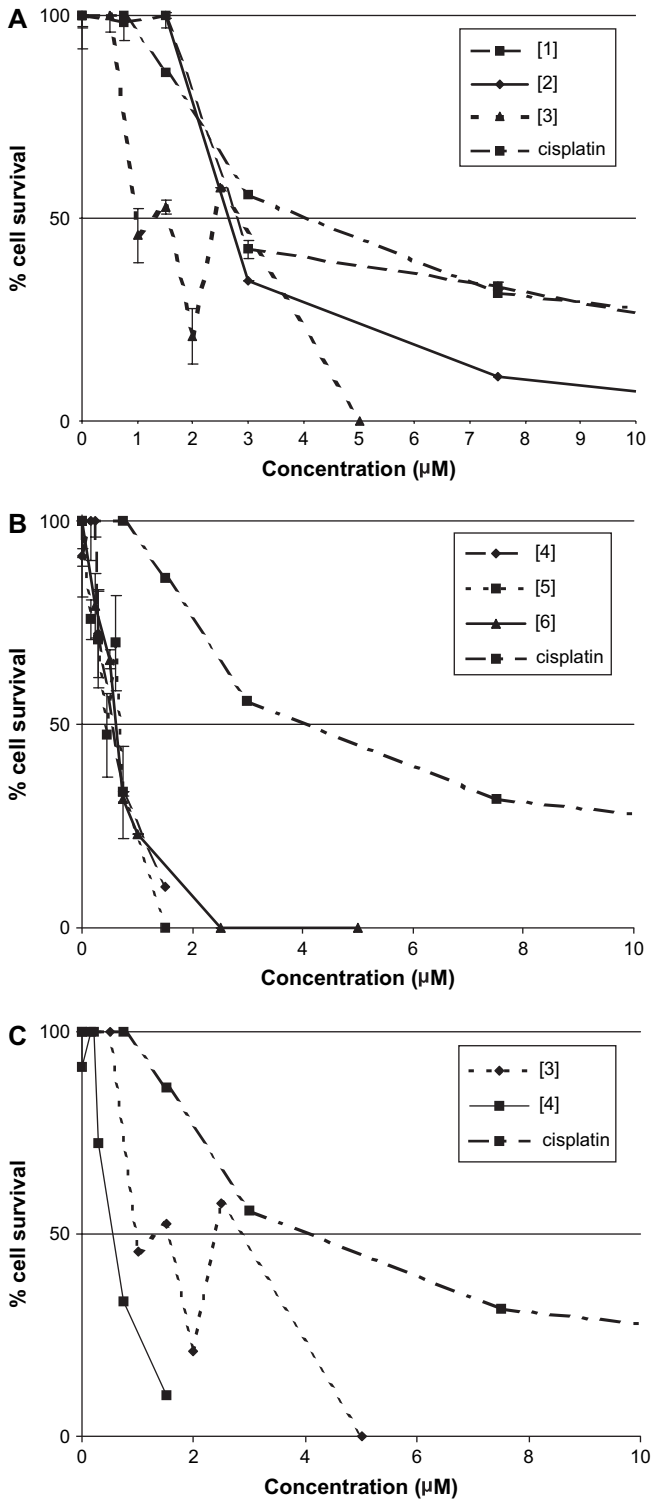


Fig. 8. % Cancerous cell survival in the presence of various concentrations (μM) of complexes (**1**)–(**6**) in contrast to those of cisplatin [6b]. Six replications per treatment group for the cytotoxic experiments were used. (A): % cancerous cell survival in the presence of triphenyltin(IV) complexes (**1**)–(**3**) in comparison with the corresponding in the presence of cisplatin. (B): % cancerous cell survival in the presence di-organotin(IV) complexes (**4**)–(**6**) in comparison with the corresponding in the presence cisplatin. (C): Comparison between the complexes with strongest cytotoxicity from the tri- and di-organotin complex groups.

the ligands of the complexes, R_2SnL_2 complexes are expected to exhibit stronger activity as oxidation promoters or enzyme inhibitors and therefore possess better cytotoxic activity, although they have no coordination position available.

5. Experimental

5.1. Materials and methods

All solvents used were of reagent grade, while thioamides and organotin chlorides (Aldrich, Merck) were used with no further purification. Diphenyltin oxide was prepared by reacting diphenyltin dichloride with potassium hydroxide as described previously [6a]. Elemental analysis for C, H, N, and S were carried out with a Carlo Erba EA MODEL 1108. Infra-red spectra in the region of 4000–370 cm^{-1} were obtained in KBr discs.

5.2. Preparation of complexes (*1*)–(*6*)

Compounds (**1**)–(**6**) were prepared following methods described elsewhere [6a,15,16].

5.3. The influence of organotin(IV) complexes (*3*)–(*6*) upon the peroxidation of oleic acid

Oleic acid, 18:1(Δ9), (Sigma, 99%) was used as supplied. The monitoring of oleic acid peroxidation level was performed by the determination of the total concentration of the isomeric hydroperoxides R′OOH using iodometric titration. The oxidation of constant volume of the acid (5 ml) was carried out in a thermostatic cell using an air flow with the constant rate 2–4 ml/min at 65 °C and 37 °C. At 65 °C the oxidation proceeds in the ''kinetic range'' under these conditions, the oxidation rate is independent of air volume passing through the cell [18a]. The temperature 37 °C was chosen to model the physiological conditions of the lipid fragment's peroxidation. Since the oxidation proceeds as an auto-oxidation, the air flow has been passed through oleic acid during 2 h before addition of the compounds under investigation. The concentrations of the additives R_nSnL_m were 1 mmol l^{-1} compared with the initial concentration of hydroperoxides in the reaction mixture. The rate of hydroperoxides' accumulation was determined by kinetic measurements. The approximation coefficients of kinetic curves were in the range of 0.9650–0.984. The kinetic curves of the acid oxidation in the presence of organotin complexes follow the exponential law. Kinetic investigation has shown the initial rate of the hydroperoxides' accumulation to be pseudo first-order in air.

5.4. Study of lipoxygenase inhibition mechanism

5.4.1. Preparation of solutions

The buffer used for all experiments was 0.2 M boric acid at pH 9: 0.1 mol boric acid (H_3BO_3, 6.18 g) was added to 300 cm^3 distilled water. The pH was adjusted to 9 with 50% w/v NaOH. Finally the solution was diluted to 500 cm^3

[21a]. Linoleic acid substrate solution was prepared as described below: 0.05 cm^3 of linoleic acid was dissolved in 0.05 cm^3 95% ethanol in a volumetric flask (50 cm^3). The appropriate volume of H_2O was gradually added in the flask. To 30 cm^3 of the borate buffer, 5 cm^3 of the solution prepared was added. Enzyme solution (lipoxygenase): a solution of 10 000 units of enzyme for each cubic centimeter of borate buffer was prepared in ice cold bath [21a]. An amount of 500 units for every 3 cm^3 of reaction mixture is used in every experiment. A unit of lipoxygenase causes an increase in absorption at 234 nm equal to 0.001 per minute.

5.4.2. Procedure

Enzyme activity was monitored by UV analysis. Enzyme solution (0.05 cm^3) was added to a cuvette containing 2 cm^3 linoleic acid solution and the appropriate amounts of buffer and inhibitor solutions in thermostatic water bath at 25 °C. There was no pre-incubation time of the enzyme with inhibitor solution. The activity of the enzyme was determined by monitoring the increase in the absorption caused by the oxidation of linoleic acid at 234 nm and 25 °C [21b] ($\varepsilon = 25\,000\ M^{-1}\,cm^{-1}$ [21c,21d]). Four standard solutions of complexes in DMSO (10^{-2}, 5×10^{-3}, 2.5×10^{-3}, 10^{-3} M) were used for the inhibition activity experiments (three sets). In this case, the substrate concentration was kept constant (0.3 mM), while the amounts of buffer and inhibitor solutions varied according to the inhibitor final concentration needed (5–60 μM or 9–18 μl from standard solutions). The total experiment volume was 3 cm^3.

For K_m and V_{max} determination experiments (three sets of experiments), the concentration of inhibitor was kept constant (15 μM, 9 μl from a standard solution of 5×10^{-3} M in DMSO) and the substrate concentrations used were 0.01 mM, 0.025 mM 0.05 mM, 0.075 mM and 0.1 mM. All solutions were kept at thermostatic water bath at 25 °C, except for the enzyme solution that was kept at ice cold bath (0 °C).

5.5. Computational methods – docking study

The docking program used was ArgusLab [22a]. The program was also utilized for the visualization and molecular modeling of the compounds. The three dimensional coordinates of lipoxygenase were obtained through the Internet at the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank [22b] and of the complexes by X-ray diffraction analysis [6a]. ArgusLab implements an efficient grid-based docking algorithm, which approximates an exhaustive search within the free volume of the binding site cavity. In order to explore the conformational space, the program performs a geometry optimization fit of the flexible ligand (rings are treated as rigid) along with incremental construction of the ligand's torsions. Thus, docking occurs between flexible ligands and a rigid protein. The orientation of the ligand is evaluated with a shape scoring function based on an enhancement of the XScore(HP) method of Wang and coworkers [22c] and final poses are ranked by lowest interaction energy values. Prior to docking, ground state optimizations on the X-ray structures of the complexes were carried out using the PM3 parameterization scheme [22d] as implemented in the ArgusLab package in order to confirm no significant divergence in conformation of the complexes due to crystal packing effects.

5.6. Biological tests

Measurements of *in vitro* cells' toxicity have been carried out in preliminary repetitions. Cell proliferation/survival was measured (%) at 24 h and 48 h of exposure. Cell lines were maintained in Dulbecco's modified eagle medium (DMEM) with 10% fetal calf serum (FCS), incubated at 37 °C, 5% CO_2. The test compounds, were each weighted and dissolved to equal a 1 μM solution. From this solution a dilution was made into the test range concentration of 1 μM down to 0.025 μM. Exponentially growing cancer cells were seeded in 3.5 cm, 6 well culture plates at 50 000/ml and allowed a 24-h doubling time and then treated with 100 μl of various concentrations of tested compounds. After 24 h and 48 h the cells were washed with buffer solutions, detached from support with trypsin–EDTA and counted on hemocytometer (Bauer slide) [23].

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References

[1] B. Rosenberg, V. van Camp, J.E. Trosko, V.H. Mansour, Nature 222 (1969) 385–386.
[2] M. Gielen, Appl. Organomet Chem. 16 (2002) 481–494 and references therein.
[3] (a) P.J. Loehrer, L.H. Einhorn, Ann. Int. Med. 100 (1984) 704–713;
(b) J.C. Ruckdeschel, Semin. Oncol. 21 (5, Suppl. 12) (1994) 114–118.
[4] R.B. Weiss, M.C. Christian, Drugs 46 (1993) 360–377.
[5] N. Cardarelli, S.V. Kanakkanatt, U.S. Patent Publ., U.S. 4,541,956, 1985.
[6] (a) M.N. Xanthopoulou, S.K. Hadjikakou, N. Hadjiliadis, M. Schürmann, K. Jurkschat, A. Michaelides, S. Skoulika, T. Bakas, J.J. Binolis, S. Karkabounas, K. Charalabopoulos, J. Inorg. Biochem. 96 (2003) 425–434;
(b) M.N. Xanthopoulou, S.K. Hadjikakou, N. Hadjiliadis, M. Schürmann, K. Jurkschat, J.J. Binolis, S. Karkabounas, K. Charalabopoulos, Bioinorg. Chem. Appl. 1 (2003) 227–231;
(c) L. Pellerito, L. Nagy, Coord. Chem. Rev 224 (2002) 111;
(d) P. Yang, M. Guo, Coord. Chem. Rev. 185–186 (1999) 189–211;

(e) M. Gielen, Coord. Chem. Rev. 151 (1996) 41–51;
(f) V. Narayanan, M. Nasr, K.D. Paull, in: M. Gielen (Ed.), Tin-based Anti-tumor Drugs, Springer-Verlag, Berlin, 1990, pp. 201–217;
(g) A.K. Saxena, F. Huber, Coord. Chem. Rev. 95 (1989) 109–123;
(h) F. Huber, R. Schmiedgen, M. Schürmann, R. Barbieri, G. Ruisi, A. Silvestri, Appl. Organomet. Chem. 11 (1997) 869–888;
(i) R. Schmiedgen, F. Huber, A. Silvestri, G. Ruisi, M. Rossi, R. Barbieri, Appl. Organomet. Chem. 12 (1998) 861–871.

[7] M. Nath, S. Pokharia, X. Song, G. Eng, M. Gielen, M. Kemmer, M. Biesemans, R. Willem, D. de Vos, Appl. Organomet. Chem. 17 (2003) 305–314 and references therein.

[8] (a) J.J. Roberts, J.M. Pascoe, Nature 235 (1972) 282–284;
(b) D.R. Williams, Educ. Chem. 11 (1974) 124–127.

[9] (a) L. Ghys, M. Biesemans, M. Gielen, A. Garoufis, N. Hadjiliadis, R. Willem, J.C. Martins, Eur. J. Inorg. Chem. (2000) 513–522;
(b) Z. Yang, T. Bakas, A. Sanchez-Diaz, K. Charalabopoulos, J. Tsangaris, N. Hadjiliadis, J. Inorg. Biochem. 72 (1998) 133–140.

[10] (a) M.S. Rose, Biochem. J. 111 (1969) 129–137;
(b) M.S. Rose, E.A. Lock, Biochem. J. 120 (1970) 151–157;
(c) B.G. Farrow, A.P. Dawson, Eur. J. Biochem. 86 (1978) 85–95.

[11] (a) B. Samuelsson, S.E. Dahlen, J. Lindgren, C.A. Rouzer, C.N. Serhan, Science 237 (1987) 1171–1176;
(b) M.J. Knapp, J.P. Klinman, Biochemistry 42 (2003) 11466–11475.

[12] (a) S. Zha, V. Yegnasubramanian, W.G. Nelson, W.B. Isaacs, A.M. De Marzo, Cancer Lett. 215 (2004) 1–20;
(b) M. Kreich, P. Claus, Angew. Chem. Int. Ed. 44 (2005) 7800–7804.

[13] (a) S.J. Stohs, D. Bagchi, Free Radic. Biol. Med. 18 (1995) 321–336;
(b) T.A. Sarafian, in: A. Sigel, H. Sigel (Eds.), Metal ions in biological systems, 36, Marcel Dekker, New York, 1999, pp. 415–444;
(c) A. Gennari, B. Viviani, C.L. Galli, M. Marinovich, R. Pieters, E. Corsini, Toxicol. Appl. Pharmacol. 169 (2000) 185–190.

[14] (a) E.R. Milaeva, V.S. Petrosyan, N.T. Berberova, Yu.T. Pimenov, L. Pellerito, Bioinorg. Chem. Appl. 2 (2004) 69–91;
(b) V.S. Petrosyan, E.R. Milaeva, Y.A. Gracheva, E.V. Grigorev, V.Y. Tyurin, Y.T. Pimenov, N.T. Berberova, Appl. Organomet. Chem. 16 (2002) 655–659;
(c) A.G. Davies, Organotin Chemistry, Wiley-VCH, New York, 1997.

[15] M.N. Xanthopoulou, S.K. Hadjikakou, N. Hadjiliadis, M. Kubicki, S. Skoulika, T. Bakas, Inorg. Chem. 46 (2007) 1187–1195.

[16] (a) C.V.R. de Moura, A.P.G. de Sousa, R.M. Silva, A. Abras, M. Horner, A.J. Bortoluzzi, C.A.L. Filgueiras, J.L. Wardell, Polyhedron 18 (1999) 2961–2969;
(b) J. Susperregui, M. Bayle, J.M. Leger, G. Deleris, J. Organomet. Chem. 556 (1998) 105–110.

[17] A. Evangelou, S. Karkabounas, G. Kalpouzos, M. Malamas, R. Liasko, D. Stefanou, A.T. Vlahos, T.A. Kabanos, Cancer Lett. 119 (1997) 221–225.

[18] (a) N.M. Emanuel, E.T. Denisov, Z.K. Maizus, Liquid Phase Oxidation of Hydrocarbons, Plenum Press, New York, 1967;
(b) in: E. Niki, W. Ando (Eds.), Organic Peroxides, John Wiley, New York, 1992.

[19] (a) M.N. Xanthopoulou, S.K. Hadjikakou, N. Hadjiliadis, M. Kubicki, S. Karkabounas, K. Charalabopoulos, N. Kourkoumelis, T. Bakas, J. Organomet. Chem. 691 (2006) 1780–1789;
(b) I.H. Segel, Biochemical Calculations, second ed. J. Wiley & Sons Inc., NY, 1976.

[20] (a) T.A. Binkowski, S. Naghibzadeh, J. Liang, Nucleic Acids Res. 31 (2003) 3352–3355;
(b) J. Liang, H. Edelsbrunner, C. Woodward, Protein Sci. 7 (1998) 1884–1897.

[21] (a) A.L. Tappel, W.O. Lumdberg, P.D. Boyer, Arch. Biochim. Biophys. 42 (1953) 293–304;
(b) R.T. Holman, Methods of Biochemical Analysis, vol. 2, Interscience, NY, 1958, pp. 113–119;
(c) J.M. Lopez-Nicolas, R. Bru, A. Sanchez-Ferrer, F. Carcia-Carmona, Anal. Biochem. 221 (1994) 410–415;
(d) B. Axelrod, T.M. Cheesbrough, S. Laakso, Methods Enzymol. 71 (1981) 441–451.

[22] (a) ArgusLab, M.A. Thompson, Planaria Software, Seattle, WA 98155;
(b) Protein Data Bank, <http://www.rcsb.org/pdb/>;
(c) R. Wang, L. Lai, S. Wang, J. Comput. Aided Mol. Des. 16 (2002) 11–26;
(d) J.J.P. Stewart, J. Comput. Chem. 10 (1989) 209–220.

[23] I.R. Freshney, Culture of Animal Cells: A Manual of Basic Techniques, Alan R Liss, Inc., New York, 1987.