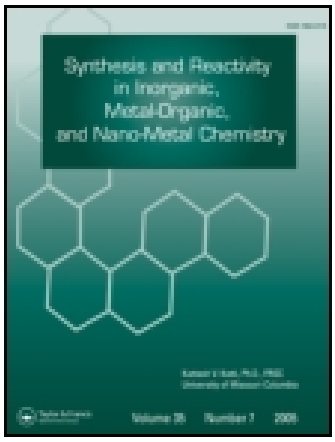

Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry

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Synthesis, Structure and In vitro Biological Activity of New Hydroxy-Naphthoquinonato Triorganotin Compounds

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Synthesis, Structure and In vitro Biological Activity of New Hydroxy-Naphthoquinonato Triorganotin Compounds

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We report herein on the synthesis, structure and in vitro antitumor activity of new triorganotin compounds of the general type $(R_3Sn)_nL$, where R = Me, Bu, Ph, Bz; L^1 = 5-Hydroxy-1,4-naphthoquinone; L^2 = 2-Hydroxy-1,4-naphthoquinone; L^3 = 5,8-dihydroxy-1,4-naphthoquinone; n = 1 for L^1 & L^2 n = 2 for L^2 and L^3. The compounds were synthesized by reacting the triorganotin hydroxide with the parent hydroxyquinone and were characterized by IR, ^{1}H-, NMR, and thermal measurements. The spectroscopic analysis provides evidence on the formation of a chelate ring that is responsible for the stabilization of the triorganotin cation with the Sn central atom in a five-coordinated environment exhibiting distorted trigonal bipyramidal geometry. The new compounds were tested for their cytotoxicity against five human tumor cell lines and one non-tumor human cell line and the results are reported.

Keywords hydroxy-naphthoquinones, triorganotins, SRB assay

INTRODUCTION

The 1,4-naphthoquinone structure is common in numerous natural products associated with antifungal, antibacterial, antiviral, and antitumour activities.[1] 1,4-naphthoquinone pharmacophore is known to impart cytotoxity in a number of drugs including among others streptonigrin,[2] actinomycins,[3] mitomycins,[4] alkannins[5] and 2-hydroxynaphthoquinone derivatives.[6]

The antitumor potential of quinonoidal compounds was initially brought up by the 1974 NCI report on the screening of 1500 synthetic and natural quinones for their anticancer activities.[7] The binding ability of quinones in different oxidation states is an additional factor for the extensive interaction of naphthoquinones with biological systems mainly through electron transfer and redox reactions.

Three major representatives of hydroxy-naphthoquinones, based on their biological activity, are juglone, lawsone and naphthazarine (Figure 1). Juglone displays potent biological properties including antimalarial, antibacterial, antiviral, and antifungal properties as well as cytotoxic properties.[8–12] Juglone's isomer, lawsone, is a natural p-quinone whose hydroxyl group in position 2, upon deprotonation, presents tautomeric structures which provide an o-quinone character that may imitate biological systems processes. Therefore, lawsone has been reported to exhibit antioxidant and immunomodulatory properties[13] as well as antimicrobial activity.[14] Due to its tautomeric effects, lawsone has been reported to form complexes with a wide range of metals.[15–18] Supporting the above, our group has previously described a number of juglone and lawsone chelates with several transition metals[19,20] that have been found to exert noticeable antimicrobial and anti-bacterial activity. On the other hand, literature on the naphthazarine nucleus is always in respect to its tautomeric character behaving as a bis-alkylating molecule. We have already reported on a cis-platin analogue naphthazarine complex with the stoicheiometry $Pt_2(C_{10}H_4O_4)(NH_3)_2Cl_2$ which exhibits potent antitumor activity comparable with that of cisplatin, but with much lower nephrotoxicity.[21] We have also noticed antibacterial and cytogenetic effects on human lymphocytes attributed to diplatinum complexes of naphthazarine.[23,23]

Continuing our work on quinone chemistry, we are now expanding our research in the study of organotin naphthoquinonates based on the literature indicating the solid biological

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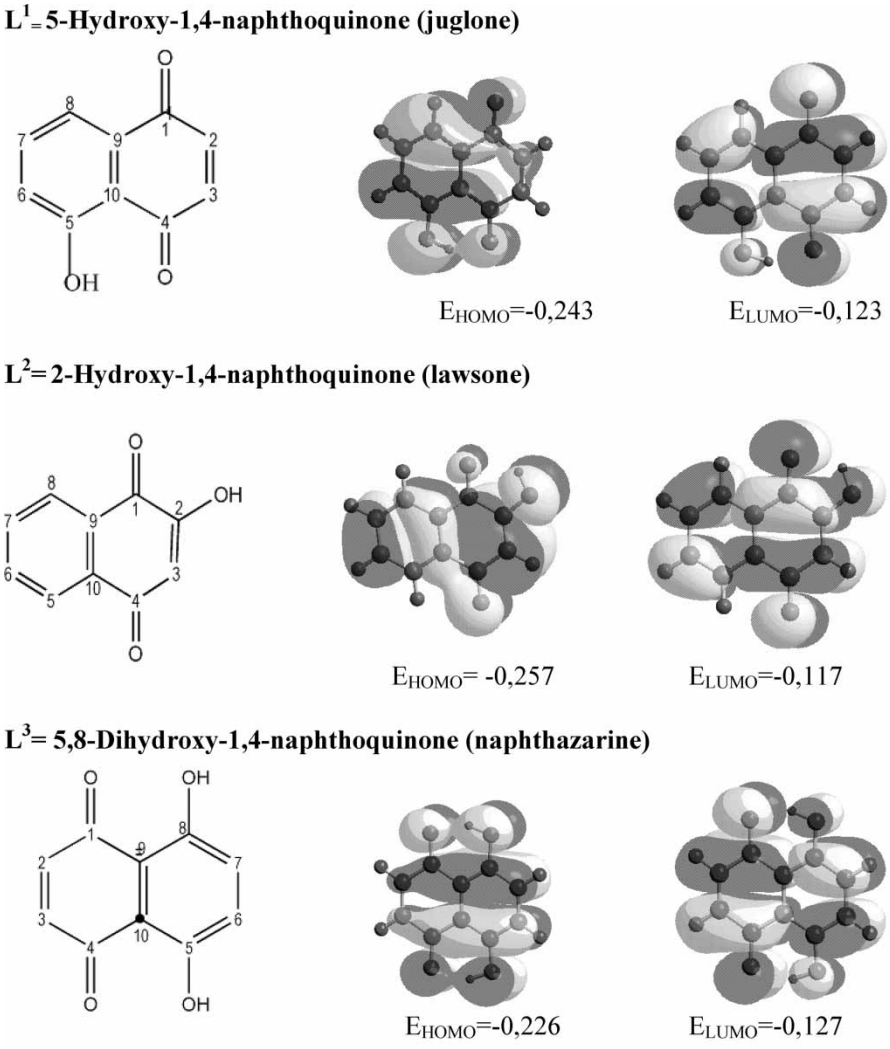

FIG. 1. (a) Structure of the hydroxy-naphthoquinones studied in this work; (b) HOMO & LUMO orbitals of the free ligands.

applications of organotin compounds. In the last 30 years, a respectful number of organotins have been synthesized, characterized and tested for their antitumor profile. The anticancer properties of more than 200 compounds from this field have been reviewed by M. Gielen.[24–28]

In general, triorganotin compounds exhibit biological activity mainly because of their ability to bind proteins.[29]

Both the structure and anti-cancer activity of triorganotins varies according to the ligand-metal interactions. The conducted studies indicate that ligands tend bind to organotin (IV) cations via monodentate, bridging, or chelating coordination modes.

Despite the detailed studies conducted for both quinones and triorganotins, only a very limited amount of work has been done to investigate the triorganotin compounds of hydroxy-naphthoquinones. Brown et al[30] has studied the organotin (IV) –orthoquinone systems and has concluded on the reaction conditions and coordination modes of benzoquinone derivatives with diphenyl- and triphenyltin.

EXPERIMENTAL

Materials. Triorganotin chlorides and hydroxy-naphthoquinones are commercially available from Sigma-Aldrich and were used without further purification. Tribenzyltin chloride was prepared by a standard method reported in the literature.[31]

All the solvents used in the reactions were of AR grade and were purchased from Sigma Aldrich.

Measurements. Carbon and hydrogen elemental analyses were performed with a Perkin-Elmer 240 B analyzer. The melting points were obtained from a Buchi B-540 model that has a maximum of 410°C. IR spectra from 4000–200 cm^{-1}

were recorded on Perkin-Elmer Spectrum One spectrophotometer with samples investigated in KBr disc in a 2:100 ratio. ^{1}H-NMR spectra were recorded with a Bruker AM 300 (300 MHz) in ca. 5% solution of $CDCl_3$ using Me_4Si as internal standard. The spectra were acquired at room temperature (298 K). Simultaneous thermogravimetric (TG) and differential thermal analysis (DTA) of the new triorganotin samples were carried out on a Setavam SetSys-12 Model instrument and spectra recording and analysis was conducted via the SetSys 2000 software. Heat was applied at the rate of 10°C/min, the temperature maximum being 650°C. The experiment was conducted under nitrogen atmosphere. Sample weights were approximately 10 mg.

Computational Details (Figures 1 and 4). To depict Figures 1b and 4, standard ab initio molecular orbital theory and DFT was used on the GAUSSIAN-98 program suite on an Intel CELERON 3,2 GHz PC.[45] The geometry of the trimethyltin derivative of juglone (compound 1; Figure 4) was fully optimized at the B3LYP level of density functional theory, using the B3LYP/LANL2DZ basis set. DFT calculations were performed on hydroxy-naphthoquinones by the hybrid B3LYP method, using the RB3LYP/6-31G(d) basis set (Figure 1). The HOMO, LUMO orbitals and the MEP surface (Figures 1 and 4) were visualized with ChemOffice 2002 (PC version).

Cell Lines. Five human tumour cell lines and one human non-tumour cell line were used in the study: K-562 (Chronic myelogenous leukemia), MCF-7 (Breast adenocarcinoma, estrogen receptor positive, ER +), HeLaS3 (Epitheloid carcinoma of cervix), PC-3 (Prostate cancer), Hs 294T (Melanoma, metastatic to lymph node) and MRC-5 (Lung foetal fibroblasts). The cells were grown in RPMI 1640 (K562 and Hs294T cells) or Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose (MCF-7, PC3, HeLaS3, and MRC5 cells). Media were supplemented with 10% of fetal calf serum (FCS, NIVNS) and antibiotics: 100 IU/ml of penicillin and 100 mg/ml of streptomycin (ICN Galenika). All cell lines were cultured in flasks (Costar, 25 cm^2) at 37°C in the 100% humidity atmosphere and 5% of CO_2. Only viable cells were used in the assays. Viability was determined using Trypan blue in a due exclusion assay.

SRB assay. Cytotoxicity was evaluated by colorimetric SRB assay after Skehan et al.[32] Single cell suspension was plated into 96-well microtitar plates (Costar, flat bottom): 1×10^4 of K562 and 5×10^3 of MCF-7, PC3, HeLa, Hs294T and MRC5 cells, per 180 ml of medium. Plates were preincubated 24 h at 37°C, 5% CO_2. Tested substances were added to all wells except the control wells. The microplates were incubated for 48 hours. After the incubation period, SRB assay was carried out: 50 μl of 80% trichloroacetic acid (TCA) was added to all the wells. An hour later, plates were washed with distillate water and 75 μl of 0.4% SRB was added to all the wells. Half an hour later, plates were washed with citric acid (1%) and dried at room temperature. Finally, 200 μl of 10 mmol TRIS (pH = 10.5) base was added to all the wells. Optical density was measured on a microplate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells, containing compete medium only, acted as the blank. Cytotoxicity was calculated according to the formula:

$$(1 - OD_{TEST}/OD_{CONTROL}) \times 100.$$

and was expressed as a percent of cytotoxicity (CI %). Data presented herein, represent the mean of the quadruplicate wells obtained from two independent experiments. IC_{50} values define the dose of compound's dose that inhibits cell growth by 50%. The IC_{50} of compounds was determined by median effect analysis and were calculated using the CalcuSyn program.

Synthesis

Juglone derivatives (compounds 1–4). 1,2 mmol of trimethyl (0-24 g), tributyl (0-52 ml), triphenyl (0.46 g), and tribenzyl tin chloride (0.51 g) accordingly were dissolved in 20 ml MeOH. The stoicheiometric quantity of NaOH was added and the resulting mixture was left stirring for about 15 minutes. Then 1 mmol (0.17 g) of the hydroxy-naphthoquinone suspended in 30 ml MeOH was added slowly, under continuous magnetic stirring, to the resulting triorganotin hydroxides. The mixtures were stirred for 24 hours at room temperature and were then condensed via evaporation. After cooling down to room temperature, the mixture was filtered. The solvent of the filtrate was gradually removed by evaporation under vacuum until solid product was obtained. The precipitate was washed several times with water, methanol, and diethyl ether. The solid was then recrystallized from ethanol. The final product was air-dried.

(1) **$SnMe_3L^1$:** dark-brown colour, Yield: 79% Anal Cal for $C_{13}H_{14}O_3Sn$ (MW = 337); C, 46,29; H,4,15%. Found: C, 46,59; H, 3,95H%.

IR(cm^{-1}): 3015–3065 m(C-Har); 2870–2945w(-C-Haliph); 1615vs(C═O); 1588s (C═C-C-O); 1259s(C-O); 587s, 560s(Sn-C); 471 m, 457s(Sn-O).

^{1}H-NMR(δ): 0.56(s) (9H: Sn-CH_3); 6,7(s) (2H: H_2, H_3); 7,2-7,8(m) (3H; H_6, H_7, H_8).

(2) **$SnBu_3L^1$:** dark-brown colour, Yield: 76%. Anal Cal for $C_{22}H_{32}O_3Sn$ (MW = 463); C, 57,02; H, 6,90%. Found: C, 57,45; H, 6,49%.

IR(cm^{-1}): 3015–3065 m(C-Har); 2920-2980 m(-C-Haliph); 1617vs(C═O); 1590s(C═C-C-O); 1264s(C-O); 507 m, 610 m(Sn-C); 220 m, 420 m(Sn-O).

^{1}H-NMR(δ): 0,9–1,65(m) (27H:Sn-Bu): 0,9(m):H_d, 1,25(m):H_c, 1,35(m):H_b, 1,65(m):H_a; 6,7(s) (2H: H_2, H_3); 7,2–7,5 (m) (3H:H_6, H_7, H_8). Numbering for the t-Bu group: $^dCH_3^cCH_2^bCH_2^aCH_2$-Sn

(3) **$SnPh_3L^1$:** dark-brown colour, Yield: 72%. Anal. Calcd. for $C_{28}H_{20}O_3Sn$ (MW = 523); C, 64,24; H, 3,82%.

Found: C, 63,97; H, 3,79%.

IR(cm^{-1}): 3015–3065 m(C-Har); 1634vs (C═O); 1595s(C═C-C-O); 1266s(C-O); 230 m, 270 m, 450vs(Sn-C); 450vs, 403 m(Sn-O).

^{1}H-NMR(δ): 6,8(s) (2H: H_2, H_3); 7.2–7.8(m) (18H: ligand and Sn-Ph protons undistinguishable)

(4) **$SnBz_3L^1$:** dark-brown colour, Yield: 71%. Anal Cal for $C_{31}H_{26}O_3Sn$ (MW = 565); C, 65,84; H, 4.60%. Found: C, 65,50; H, 4,89%.

IR(cm^{-1}): 3000-3173 m(C-Har); 2910-3000(-C-Haliph); 1643vs(C═O); 1594s(C═C-C-O); 1265 s(C-O); 239 w, 340 m, 459s (Sn-C); 440 m(Sn-O).

^{1}H-NMR(δ): 2,36(s) (6H: $-CH_2$ of the benzyl group); 6,7–6.9(s) (2H; H_2, H_3); 7,0–8,0(m) (18H: ligand and Sn-Ph protons undistinguishable)

Lawsone derivatives (compounds 5–8): The same method and quantities were applied as described here.

(5) **$SnMe_3L^2$:** dark red colour, Yield: 74%. $C_{13}H_{14}O_3Sn$ (MW = 337); C, 46,29; H, 4,15%. Found: C, 46,58; H, 3,95%.

IR(cm^{-1}): 3015–3075 m(C-Har); 2870–2945w(-C-Haliph); 1625vs(C═O); 1520s(C═C-C-O); 1261s(C-O); 558, 550s(Sn-C); 476s 419w (Sn-O).

^{1}H-NMR(δ): 0,60(s) (9H: Sn-CH_3); 6,35(s) (1H: H_3) 7,0–7,7(m) (4H: H_5, H_6, H_7, H_8)

(6) **$SnBu_3L^2$:** dark red colour, Yield: 71%. Anal. Calcd. for $C_{22}H_{32}O_3Sn$ (MW = 463); C, 57,02; H, 6,90%. Found: C, 57,15; H, 6,48%.

IR(cm^{-1}): 3015-3070 m(C-Har); 2890-2970 m(-C-Haliph); 1620vs(C═O); 1540s(C═C-C-O); 1255s(C-O); 510s, 617s(Sn-C); 225 m, 457 m(Sn-O).

^{1}H-NMR(δ): 0,9–2(m) (27H; Sn-Bu): 0,90(t):H_d, 0,95(t):H_c, 1,4(m):H_b, 2,0(m):H_a, 6,5(s): (1H; H_3); 7,26–8(m) (4H: H_5, H_6, H_7, H_8).

(7) **$SnPh_3L^2$:** dark-red colour, Yield: 78%. Anal. Calcd. for $C_{28}H_{20}O_3Sn$ (MW = 523); C, 64,24; H, 3,82%. Found: C, 63,98; H, 4,01%.

IR(cm^{-1}): 3064–3150 m(C-Har); 1613vs(C═O); 1553s(C═C-C-O); 1279s(C-O); 238 m, 277 m 451s(Sn-C); 461vs 421 m(Sn-O).

^{1}H-NMR(δ): 6,65(s):(1H:H_3); 7–8,2(m) (19H-ligand and Sn-Ph-protons, undistinguishable).

(8) **$SnBz_3L^2$:** dark red colour, Yield: 70%. Anal. Calcd. for $C_{31}H_{26}O_3Sn$ (MW = 565); C, 65,84; H, 4,60%. Found: C, 66,05; H, 4,31%.

IR(cm^{-1}): 3000–3116 m(C-Har); 2900-3000 m(-C-Haliph); 1596vs(C═O); 1509s(C═C-C-O); 1247s(C-O); 239 w, 410 w, 561 s(Sn-C); 340 m, 310 m(Sn-O).

^{1}H-NMR(δ): 2,40(s) (6H: -CH_2 of the benzyl group); 6,7(s): (1H:H_3); 7,2–7,9(m): (19H: ligand and Sn-Ph protons, undistinguishable).

Naphthazarine derivatives (compounds 9-12): The above method was applied using a 1:2,2 ratio (5,8-dihydroxy-1,4-naphthoquinone to triorganotin chloride).

(9) **$(SnMe_3)_2L^3$:** Blue-purple colour, Yield: 82%. Anal. Calcd. for $C_{16}H_{22}O_4Sn_2$ (MW = 516); C, 37,21; H, 4,20%. Found: C, 36,95; H, 3,96%.

IR(cm^{-1}): 3020–3062 m(C-Har); 2870–2950w(-C-Haliph); 1605vs(C═O); 1530s(C═C-C-O); 1175s(C-O); 570 m, 545 w(Sn-C); 440 m, 370 w(Sn-O).

^{1}H-NMR(δ): 0,65(s) (18H; Sn-CH_3); 6,8–6,9(s) (2H: H_2, H_3); 7,6(d) (2H: H_6,H_7)

(10) **$(SnBu_3)_2L^3$:** Blue-purple colour, Yield: 79%. Anal. Calcd. for $C_{34}H_{58}O_4Sn_2$ (MW = 768); C, 53.12; H, 7.55%. Found: C, 52.88; H, 7.25%.

IR(cm^{-1}): 3020–3060 m(C-Har); 2870–2960 m(-C-Haliph); 1610vs(C═O); 1547s(C═C-C-O); 1182s(C-O); 510 m, 605s (Sn-C); 410 m, 419 m(Sn-O).

^{1}H-NMR(δ): 0.8–1.8(m): (54H; Sn-Bu): 0.8(t):Hd, 1,27(m):H_c, 1,45(m):H_b, 1,8(m):H_a; 6.85 (2H:H_2, H_3); 7,45(s) (2H: H_6, H_7)

(11) **$(SnPh_3)_2L^3$:** Blue-purple colour, Yield: 87%. Anal. Calcd. for $C_{46}H_{34}O_4Sn_2$ (MW = 888); C, 62,16; H, 3,82%. Found: C, 62,49; H, 3,53%.

IR(cm^{-1}): 3013 m-3100 m(C-Har); 1608vs(C═O); 1548s(C═C-C-O); 1182s(C-O); 234 m 275 m, 457vs(Sn-C); 385 m, 470s(Sn-O).

^{1}H-NMR(δ): 6,8(s) (2H: H_2, H_3); 7,2-7,8(m)- 32H (ligand and Sn-Ph protons, undistinguishable).

(12) **$(SnBz_3)_2L^3$:** Blue-purple colour, Yield: 83%. Anal. Calcd. for $C_{52}H_{46}O_4Sn_2$ (MW = 972); C, 64.19; H, 4.73%. Found: C, 63.90; H, 4.56%.

IR(cm^{-1}): 3020-3070 m(C-Har); 2890–3000 m(-C-Haliph); 1597vs (C═O); 1490s (C═C-C-O); 1179s (C-O); 342s, 448s (Sn-C); 464 m, 310 m (Sn-O).

^{1}H-NMR(δ): 3,20(s) (12H: $-CH_2$ of the benzyl group); 6.7–6.9(s) (2H: H_2, H_3); 7.0–8.9(m) (32H: ligand and Sn-Ph protons, undistinguishable).

RESULTS AND DISCUSSION

All the new triorganotin hydroxy-naphthoquinonates are remarkably stable in light, air, or heat exposure. They are insoluble in water and moderately soluble in $CHCl_3$, DMSO, and DMF. They are dark-colored and mostly amorphous. Ligands L^1 and L^3 react readily exhibiting instant color and texture alterations while the reaction with L^2 evolves slowly with a gradual color change. This is probably due to a different kinetic phenomenon and can be attributed to the structure of the ligand (see Figure 1).

The compounds are generally synthesized by the following two routes. The first involves the metathesis reaction between the alkalimetal salt of the quinone and the corresponding organotin halide, while the second involves the interaction of the organotin hydroxide with the parent quinone. Both synthetic pathways lead to the same final products.

The triorganotin derivatives formulated as $R_3Sn(\mu\text{-L})$ or $R_3Sn(\mu\text{-L})SnR_3$ (R =Me, Bu, Ph, Bz; L = hydroxy-naphthoquinonato ligand) may be described as the corresponding hydroxy-naphthoquinonate, in accordance with the reaction stoicheiometry, the elemental analysis and spectroscopic results. The general reaction scheme is outlined in Figure 2.

Hydroxy-naphthoquinones tend to coordinate to metal ions by different modes according to Figure 3a. Our main goal has been to determine whether the formation of a chelate ring will occur or if the final product will exhibit a monodentate Sn-O bonding. Spectroscopic data provide evidence on the formation of a chelate ring responsible for the stabilization of the triorgano-tin cation with the Sn central atom in a 5-coordinated environment exhibiting distorted trigonal bipyramidal geometry. Juglone and naphthazarine are coordinated to central Sn (IV) via a phenolic and a quinoidal oxygen forming 6-membered rings, while lawsone forms a 5-membered chelate with the participation of its phenolic oxygen and its neighboring quinoidal one (Figure 3b). The R-substituents of the tin cation complete the trigonal bipyramidal coordination environment.

Figure 4a depicts the optimized structure of trimethyltin juglonato (compound 1). It is interesting to notice that, in spite of the R-substituents attached to Sn and the steric demands of the naphthoquinonic ring, the juglonato ligand prefers to function as a chelating moiety giving out a five-membered coordinated structure.

As expected for a R_3SnO_2 structural motif, the tin(IV) atom bears a five-coordinate geometry with two of the R-substituents occupying the equatorial positions of a trigonal bipyramide. The third R-group is found on the apical site.

In a second step, the observation of the HOMO and LUMO of L^1 and its trimethyltin derivative (Figure 4b) suggest that the trimethyltin fragment is not involved in the description of the frontier orbitals and that the charge transfer remains located on the conjugated ligand as anticipated in the case of molecular chromophores (Figure 4c).

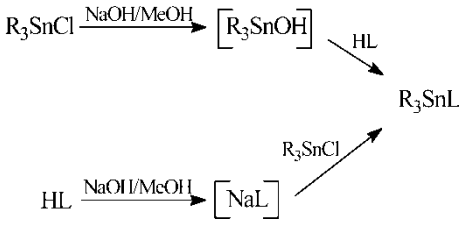

FIG. 2. General reaction scheme: Presentation of the two alternative pathways for the general preparation of triorganotin hydroxy-quinonates. The reaction scheme applies to a mono-hydroxy-naphthoquinonate (HL); in the case of bis-hydroxy-naphthoquinonates (H_2L) the triorganotin to hydroxy-quinone ratio is set to 2:1 and the compound should be referred to as $(R_3Sn)_2(\mu\text{-L})$. The above synthetic route has been applied to trimethyl, tributyl, triphenyl and tribenzyl derivatives.

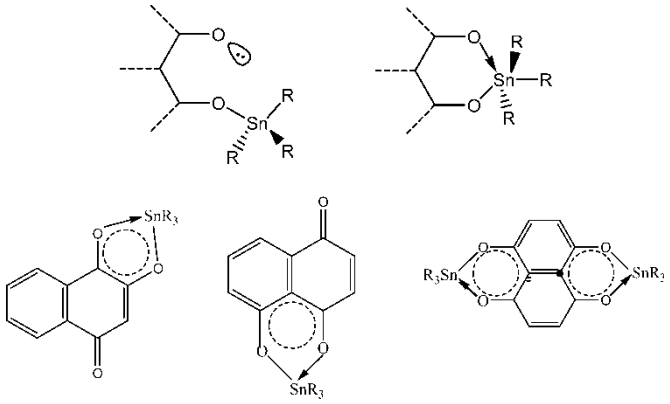

FIG. 3. (a) The two probable coordination modes of the compounds studied. The carbonyl oxygen lone pair is drawn in the tetrahedral species to account for the bond in the trigonal bipyramidal one; (b) Proposed structures of the new triorganotin naphthoquinonates.

IR Spectra

Several indications were obtained from a close comparison between the spectra of the free ligands and those of related complexes. The sharp bands in the region 3500–3000 cm^{-1} obtained in the spectra of the free hydroxy-naphthoquinones are assigned to the -OH stretching vibrations and disappear in the spectra of the new compounds. The new broad weak bands in the 3500–3300 cm^{-1} region in complexes 2, 7 and 10 were attributed to the presence of some non-coordinated water molecules in the samples.

The strong broad band, centered in the donors at $\sim$2600 cm^{-1} due to the intramolecular hydrogen bond (O-H. . .-O), disappears in the triorganotin derivatives. The ν(=C-H)stretching vibrations of the free ligands appear in the region 3010–3060 cm^{-1} and show no major spectral changes upon complex formation. In the spectra of trimethyltin complexes, the medium stretching vibrations of the methyl groups were found in the region 2870–2990 cm^{-1}. In the spectra of all tributyltin complexes, we assign the very strong stretching vibrations of the *n*-Bu skeleton at 2956 cm^{-1} for the ν_aCH_3 and at 2925 $cm^{-1}$for the (ν_aCH_2) group respectively. In the spectra of the tribenzyltin derivatives, the incorporation of the tribenzyl- group is indicated by the typical pattern present in the region 2915–3100 cm^{-1}, accounting for ν(C-C-H) and ν(C=C-H) stretching vibrations.

In the new complexes, both carbonyl vibrations are shifted to lower wavenumbers. The ν(C=O) stretching vibration of the free ligands (C_4=O for L^1, C_1=O for L^2; C_1=O and C_4=O for L^3) appears as a strong intensity band at 1664 cm^{-1} for juglone, 1675 cm^{-1} for lawsone, and 1621 cm^{-1} for naphthazarine, respectively. Upon complexation, the hydrogen-bonded carbony1 band of the free ligands is bathochromically shifted to lower frequencies by 10–80 cm^{-1}, depending on the complex corroborating the participation of the quinonic carbonyl (C=O) group in coordination

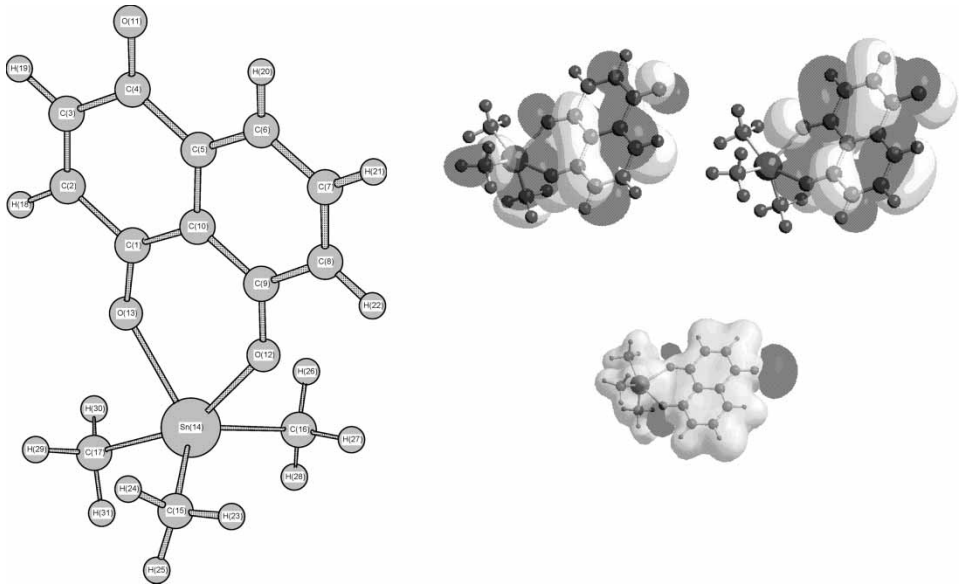

FIG. 4. (a) Optimized structure of trimethyltin juglonato (compound1) computed at the B3LYP/LANL2DZ level of theory; (b) HOMO and LUMO orbitals of trimethyltin juglonato (compound 1) at the B3LYP/LANL2DZ level of theory; (c) Molecular electrostatic potential (MEP) (isopotential surfaces of 1.00 a.u.) of juglonato trimethyltin (compound 1) at the B3LYP/LANL2DZ level of theory (white colour represents positive; black colour represents negative).

with tin. The shift in the positions of these bands is extensively used as a diagnostic tool of the hydroxy-naphthoquinonate anions' coordination mode.

The shifted carbonyl band activates the adjacent C═C skeletal in-plane vibration of the conjugated aromatic ring and these activated C═C vibrations can be seen in the region 1509–1597 cm^{-1}, at lowered wavenumbers by 10–85 cm^{-1} when compared with those of the free ligands. In the free ligands spectra, the ν(C-O) band appears in 1216 cm^{-1} for the juglonato moiety, 1226 cm^{-1} and 1142 cm^{-1} for the lawsonato and naphthazarinato moieties. Upon derivatization, due to the electronic density delocalization of the system, a shift in higher frequencies by 30–65 cm^{-1}, is noticed. This is consistent and indicative of the coordination of the phenolic or quinonic oxygen with tin.

In the complexes spectra, we assign the strong or medium absorptions in the range 470–340 cm^{-1} to symmetric and asymmetric ν(Sn-O) modes, based on the literature available on β-diketonato derivatives.[33,34] The ν(-OH) band of Ph_3SnOH (3615 cm^{-1}) is not found in the spectra of the products, indicating complex formation together with the appearance of intense -C-H out-of-plane deformation bands at ~730 and ~690 cm^{-1}. Moreover, in the spectra of the triphenyltin derivatives, the symmetric and asymmetric stretching vibrations of the $SnPh_3$ moiety, already described by Whiffen,[35] appear at ca. 450, 270 and 230 cm^{-1}. The triphenyltin compounds show the usual benzene ring absorptions. In particular, a band at ~1077 cm^{-1}, which is assigned to a C-H in plane deformation vibration, has been shown to be characteristic of the phenyltin group. In the spectra of tributyltin derivatives the Sn-Bu vibrations were observed at ca. 610 and 510 cm^{-1}. New peaks in the region 610–220 cm^{-1} are assigned to ν(Sn-O) and ν(Sn-C) stretching vibrations and also confirm the coordination. The ν(Sn-Cl) band that appears in the range 270–290 cm^{-1} of the spectra of the triorganotin chlorides, is absent in all complexes.

The above indicate the coordination of hydroxy-naphthoquinones to tin via quinoidal and phenolic oxygen forming a 6-membered chelate ring in the case of L^1 and L^3 and a 5-membered chelate for L^2.

^{1}H-NMR Spectra

The ^{1}H NMR spectra of the new complexes show the expected integration and peak multiplicities but aromatic signals undergo a more complex pattern upon chelation.

In compounds 3,4,7,8,11 and 12 a typical singlet at 1,5 δ is observed that is assigned to the water of $CHCl_3$.

In all the studied triorganotin (IV) hydroxy-naphthoquinonates the signal due to the hydrogen of phenolic or quinoidal protons of the free ligands disappears suggesting coordination with tin.

The chemical shifts of the signals for the methyl, butyl, phenyl and tribenzyl group appear at the same position as in the free organotins and have been assigned in the spectra of the complexes. More particularly, the Sn-CH_3 moiety is found at 0.56 δ for L^1, 0.60 δ for L^2 and 0.65 δ for L^3 as a single signal in all three cases. The tributyltin (IV) derivatives show at least two different sets of signals for the alkyl groups bonded to tin, which are typical for five-coordinate species. Another set of multiplets due to C-H protons is observed

between 0.80 and 2.0 δ for these derivatives. In L^1, the band is seen at 0.9–1.65 δ. In L^2 the same band is located in the region 0.9–2 δ(m). Similarly, we assign the multiplet in the area 0.8–1.8 δ for the tributyl derivative of naphthazarine. In the spectra of the triphenyltin (IV) complexes, it is not possible to distinguish between signals due to aromatic protons of the ligand and those linked to tin, but integration took their presence into account. A complicated set of multiplets due to the ligand aromatic and phenyl ring protons of the Sn-Ph_3 moiety is observed in the range 7.2–7.8 δ for compounds 3 and 11 and in the range 7–8.2 δ for compound 7.

The -CH_2 group of the tribenzyl tin moiety is present at 2.36 δ as a singlet in compound 4, at 2.40 δ also a singlet for compound 8 and at 3.20 δ corresponding to 12H for the tribenzyltin derivative of naphthazarine.

The downfield shift of the signals for H_3 and H_6 for juglone, H_6 and H_8 for lawsone and H_2, H_3, H_6 and H_7 for naphthazarine are attributed to the involvement of the corresponding quinoidal carbonyls and phenolic hydroxyls in complexation. This trend is correlated with the formation of Sn-O bonds, which generate a decrease of electron density on the hydroxy-naphthoquinone ring.

In compound 2, a singlet at 6,7 δ corresponding to two protons provides evidence on the coordination and is similar to the singlet at 6.7–6.9 δ of compound 4. H_3 and H_6 of L^1 undergo the higher shifts because of the coordination taking place in their neighbourhood. In the lawsone compounds (5–8), we have been able to distinguish the H_3 proton in the trimethyl [6,35 δ(s)], tributyl [6,5 δ(s)], triphenyl [6,65 δ(s)], and finally to the tribenzyl [6,7 δ(s)] derivative. As far as the naphthazarine triorganotins are concerned, it is noteworthy that the two protons of the quinonic ring (H_2, H_3) undergo a noticeable shift and are located at the range 6,8–6,9 δ(s) as a singlet. The poor solubility of the compounds prevented observation of the expected tin satellites.

Thermal Studies

The observations made during the acquirement of the melting points have dictated the need to further investigate the thermal profile of the new compounds. In any of the cases, we did not notice a clear melting point. On the contrary, multiple dissociations (even in temperatures around 120°C) are apparent, followed by texture and color alterations, indicating that the new complexes undergo phase alterations during their exposure to heat. The coordination course of hydroxy-naphthoquinones used in this study further support the above remarks, given their ability to exist in different oxidation states, which lead to variable final structures. The triphenyl tin naphthoquinonates were studied in a range up to 650°C, but up to this temperature, the total mass loss does not exceed the 50% of the initial mass (Table 1). This implies either that breakdown is not complete until this temperature or that SnO_2 formation occurs but organic residues remain.

Contrary to the belief that the Sn-C bond is stable at temperatures up to 200°C, evidence for decomposition is seen in forms of exothermic processes at much lower temperatures. In the compounds studied herein, several bonds could cleave at elevated temperatures e.g., Sn-C, Sn-0, C-C, C-0 and C-H. However, the Sn-C bond must be considered to be the least thermally stable. To support this, the IR spectrum of the residues, all the typical bands of the hydroxy-naphthoquinones used are still present, indicating the thermal stability of the ligands used. The thermogravimetric (TG) analysis reveals that the decomposition occurs as the temperature increases. The degradation patterns are not differentiated according to the ligand but only according to the R- substituent of the organotin moiety. The weight losses observed due to thermal decomposition are quite close to the calculated values. The slight differences in the values indicate the error, which in any case remains within the acceptable range of $\pm$3%.

TABLE 1
Thermal data of Ph_3SnL^1 and $(Ph_3Sn)_2L^3$

		TG/DTG			DTA(°C)	
Compound	Step	Temp range (°C)	Mass loss % Found (Theor)	Moiety evolved	endo (−)	exo (+)
Ph_3SnL^1	1	90–125	—	—	100	—
					125	—
	2	200–250	12,80%(14,72%)	Phenyl group	230	215
	3	275–360	415% (39,38%)	SnPh moiety	—	350
	Residue	>650 °C	—	—	—	—
$(Ph_3Sn)_2L^3$	1	75–135	1,5%(8,67%)	Phenyl group	100	—
					135	—
	2	220–250	12,5%	—	240	215
	3	275–340	38%(39,41%)	$SnPh_3$ moiety	—	325
	Residue	>650 °C	—	—	—	—

The degradation pattern of the triphenyl tin derivative of juglone and lawsone (Ph_3SnL^1, Ph_3SnL^2) are similar and show a slight weight loss of ~12% up to 250°C. In the juglone derivative, two enotherms are observed at 100°C and 125°C, followed by an exotherm at 215°C and an endotherm at 230°C. None of these phenomena can be attributed to the compounds' melting and have therefore been assigned to the typical for organotins, phase alteration processes. From the temperature of 250°C and up, a rapid weight loss begins. At 350°C, an exotherm is noticed, corresponding to 40% weight loss, which may be accounting for the SnPh moiety. The gradual weight loss continues up to 650°C without any evidence of the juglone or lawsone degradation. The triphenyl derivative of naphthazarine $(Ph_3Sn)_2L^3$, follows a three-step decomposition pattern. Initially, we notice two distinct endothermic phenomena at 100°C, and 135°C, respectively, with a weight loss around 7.5%, corresponding to the loss of one phenyl group. This is followed by an exotherm at 215°C and an endotherm at 240°C with no noticeable weight loss. This may indicate that within this temperature range, oxidative or thermal degradating processes are occurring. Between 225°C–350°C, a gradual but rapid weight loss occurs. The last step of the decomposition is characterized by an exothermic phenomenon at 325°C, which shows a 38% weight loss and probably corresponds to the evolution of one $SnPh_3$ moiety. Up to 650°C, no evidence of naphthazarine degradation is present.

Cytotoxicity Studies

A useful organotin anti-tumor agent ought to possess alkyls of low toxicity and high activity that will induce a subsequent dealkylation in vivo and will therefore promote biological activity. Such equilibria are found among the butyl and phenyl derivatives and this is why they are popularly used in the synthesis of experimental anti-tumor tin drugs.

Based on the preceding, we have measured the percent (%) of cytotoxicity of the new triphenytin compounds and two of the tributyltin derivatives, against five human tumour cell lines K562, MCF-7, HeLaS3, PC3, Hs 294T and one non-tumour human cell line MRC5. The compounds have been tested using the colorimetric SRB assay. The results are summarized in Table 2. Most of the compounds were found to be toxic not only against tumour cells but, unfortunately, against normal MRC5 cells as well. The new compounds were tested in the range of concentrations from 10^{-8} to 10^{-4} M and they were continuously present in the culture for 48 h.

Regarding the profile of cytotoxicity, dose dependent response was found for all cell lines except PC3 (prostate carcinoma cell line), i.e., cytotoxicity of compound increased with concentration, whereas in PC3 cell line the highest response was obtained with the smallest concentration of compounds. This is quite unusual but the same response was obtained during the second experimentation with PC3 cells and therefore we have concluded that this is a true response and not an experimental mistake. Due to absence of dose-response in PC3 cells, IC_{50} values were not calculated for the majority of the compounds. Additionally, we have noticed a dose-dependent response for all cell lines. Most of the compounds at concentrations of 10^{-5} and 10^{-4}M gave cytotoxicity above 80% regardless of the cell type. Another observation is that there is no tissue specific cell response, thus cells of different origin (leukemia cells, breast and cervix cancer cells, normal lung fibroblasts) are sensitive to the tested compounds.

HeLa cells appeared slightly more sensitive to all compounds when compared to other cell lines. The triphenyl and tributyl derivatives of naphthazarine are approximately 100 fold more potent than doxorubicin against HeLa cells. Simultaneously, we notice that HeLa cells are almost twice as sensitive as doxorubicin to triphenyltin lawsonato, while they show response that is analogue to the reference compound when treated with tributyltin juglonato. The melanoma cells (Hs 294T) are known to be non-sensitive to doxorubicin and we have acquired the same result for our

TABLE 2
IC_{50} (μM) values (SRB assay) of some of the new hydroxy naphthoquinonato triorganotin derivatives

	IC_{50}, μM, 48 h, SRB				
	K562	MCF 7	HeLa	Hs 294T	MRC 5
Doxorubicin[a]	0.82	1.19	5.08	29.85	0.32
Ph_3SnL^1	0.09	2.28	0.06	0.58	0.0012
Bu_3SnL^1	0.55	19.97	4.67	23.33	0.0004
Ph_3SnL^2	0.29	0.12	9.87	0.28	0.0082
$(Ph_3Sn)_2L^3$	0.38	1.46	0.04	0.59	0.06
$(Bu_3Sn)_2$-L^3	>100	>100	0.05	24.58	9.76

[a]Doxorubicin (Mol. Formula: $C_{27}H_{29}NO_{11}$) is an antibiotic belonging to the anthracycline group, widely used in human cancer chemotherapy in breast and pulmonary cancers. Its activity has been mainly attributed to its intercalation between the base pairs of DNA.

tributyltin derivatives, while at the same time a remarkable sensitivity is expressed towards the triphenyltins.

High cytotoxicity against MRC5 cells is surely undesirable and as a result, these compounds are not available candidates for antitumor testing on tumor bearing animals. The presenting data indicate that in most cases, the tributyltin derivatives are less cytotoxic than the triphenyl derivatives.

In our effort to justify the toxic profile of the tested compounds, we have attributed a key influence to the compounds' limited solubility, which does not allow cellular penetration of any kind. A successful anticancer agent ought to be soluble in non-toxic solvents to facilitate administration either to the bloodstream or to the alimentary tract. Triorganotins are reported to transport organic anions across the phospholipid bilayer through an exchange diffusion mechanism with chloride but their membrane permeability potential is in strict line with the ligand they carry.[36] In our case, the ligands are bulky enough to prevent the new compounds from entering a cell.

A definite correlation also exists between cytotoxicity and lipophilicity, with highly hydrophilic or lipophilic agents being far less toxic when compared to the intermediate species. We presume therefore, that since the toxicity of the parent R_3SnX analogues are higher than that of R_2SnX_2 or $R_2SnX\ L_2$ (where L = bidentate ligands) compounds,[37] this results in a highly toxic profile for their deriving compounds as well.

The other factor decisively affecting the toxicity in this case is the nature of the ligands. Early enough, d' Arcy-Doherty,[38] has shown that juglone is more toxic than lawsone to rat hepatocytes by an order of magnitude which has been attributed to the potential of these compounds to induce conditions of oxidative stress and depletion of reduced GSH. The cytotoxicity of these quinones is enhanced upon metal complexation. For example, the iron complexes of juglone are more toxic than the ones of lawsone indicating that the toxicity mechanisms differ. It has been suggested that the depletion of intracellular glutathione by lawsone is enzyme mediated whereas in the case of juglone, the direct chemical reaction with glutathione is responsible for the depletion. The mechanism of toxicity of juglone involves the formation of its corresponding naphthosemiquinone, active oxygen species and redox cycling. No such evidence is available for lawsone, due to its very low one-electron reduction potential (E1/7-415 mV), which offers a very poor substrate for reduction to a semiquinone by cellular reductases. As far as the effect of hydroxyl substitution on the toxicity of 1,4-naphthoquinones is concerned, the sequence of potency is 5,8-dihydroxy-1,4-naphthoquinone $>$5-hydroxy-1,4-naphthoquinone $<$2-hydroxy-1,4-naphthoquinone as Babich[39] showed in his experiments with HepG2, and BALB/cell lines.

Based on the above, the toxic profile in the case herein might in part result from the dissociation of the complexes in the cell culture media, resulting in the production of semiquinones or free radicals that will interfere with DNA replication while at the same time, the binding affinity of tin to proteins or enzymes will change their interaction process with DNA, thereby affecting cell proliferation. Although the details of the mechanism of DNA-organotin interaction have not yet been clearly defined, there is solid evidence that the majority of organotins are toxic because they combine with an enzyme inactivating it. Tin forms a bond with the active site that is too strong to be readily broken, thus preventing the enzyme from reacting with its substrates.

Organotins are known to interact in vitro with several enzymes e.g., human aromatase and acetyltransferase,[40] while there is evidence that they enhance human CG secretion as well.[41] Given that naphthoquinones are also involved in the in-vivo biosynthesis of estrogens,[42] a proposed toxicity mechanism may involve the combinatorial effect of the inhibitory action of an enzyme like cytochrome P-450 by the tin moiety acting as an endocrine-disrupting agent along with the simultaneous activity of the naphthoquinone metabolites—semiquinone radicals—that will produce hydroxyl radicals, powerful oxidizing agents that are responsible for damaging among others, the estrogen biosynthesis. This synergistic effect might be able to explain the higher sensitivity of HeLa cells given that the cervix is one of the organs where steroids are synthesized. This might also interpret the peculiar response of PC3 cells if we take into consideration the ability of organotins to interact with 5-a-reductase (the enzyme that catalyses the testosterone biosynthesis) as well as the inhibitory effect that semiquinone radicals may have in its intermediate stages.

Nevertheless, both the stability and solubility of the new triorganotins should be accounted when proposing this mechanism. Additionally to the above, we believe that the nature of the ligand environment in combination to the character of tin in the coordination process also influences the cytotoxicity pattern. Based on previous studies on structure-/activity correlation of organotins (IV),[43,44] we have attributed a role on the availability of coordination positions at tin. The cytotoxic activity of triorganotins (IV) is dependent on their geometric behavior when in solution. The more active tetrahedral structures are bound to increase their coordination number through O, S, or N donors while the five coordinated species, which is the case herein, cannot undergo further coordination and are expected to exhibit their activity as they stand. The preceding remarks combined with the proposed structure of the new compounds, should justify their toxic profile.

REFERENCES

1. O'Brien, P. J. Molecular mechanisms of quinone cytotoxicity. *Chem. Biol. Interact.* **1991**, *80*, 1–41.
2. Yee, S. B.; Pritsos, C. A. Comparison of oxygen radical generation from the reductive activation of doxorubicin, streptonigrin, and menadione by xanthine oxidase and xanthine dehydrogenase. *Arch. Biochem. Biophys.* **1997**, *347* (2), 235–241.

3. Martin, R. B. 2-amino-1,4-naphthoquinone as a model compound for actinomycins. *Acta Biochim. Biophys.* **1964**, *91*, 642–644.
4. Moore, H. W.; Czerniak, R. Naturally occurring quinones as potential bioreductive alkylating agents. *Med. Res. Rev.* **1981**, *1* (3), 249–280.
5. Papageorgiou, V. P.; Assimopoulou, A. N.; Couladouros, E. A.; Hepworth, D.; Nicolaou, K. C. The chemistry and biology of alkannin, shikonin, and related naphthazarin natural products. *Angew Chem. Int. Ed.* **1999**, *38* (3), 270–301.
6. Hatzigrigoriou, E.; Papadopoulou, M. V; Shields, D. 2-Alkylsulfonyloxy-3-hydroxy-1,4-naphthoquinones: A novel class of radio- and chemosensitizers of V79 cells. *Oncol. Res.* **1993**, *5*, 29–36.
7. Asche, C. Antitumour quinones. *Mini Rev. Med. Chem.* **2005**, *5* (5), 449–467.
8. Inbaraj, J. J.; Chignell, C. F. Cytotoxic action of juglone and plumbagin: a mechanistic study using HaCaT keratinocytes. *Chem. Res. Toxicol.* **2004**, *17*, 55–62.
9. Paulsen, M. T; Ljungman, M. The natural toxin juglone causes degradation of p53 and induces rapid H2AX phosphorylation and cell death in human fibroblasts. *Toxicol. Appl. Pharmacol.* **2005**, *209* (1), 1–9.
10. Wang, H.; Mao, Y.; Chen, A. Y. Stimulation of topoisomerase II-mediated DNA damage via a mechanism involving protein thiolation. *Biochemistry* **2001**, *40* (11), 3316–3323.
11. Chao, S. H.; Greenleaf, A. L.; Price, D. H. Juglone, an inhibitor of the peptidyl-prolyl isomerase Pin1, also directly blocks transcription. *Nucleic Acids Res.* **2001**, *29*, 767–773.
12. Varga, Z.; Bene, L.; Pieri, C.; Damjanovich, S.; Gaspar, R. Jr. The effect of juglone on the membrane potential and whole-cell K+ currents of human lymphocytes. *Biochem. Biophys. Res. Commun.* **1996**, *218* (3), 828–832.
13. Habbal, O. A.; Al-Jabri, A. A.; El-Hag, A. H.; Al-Mahrooqi, Z. H.; Al-Hashmi, N. A. In-vitro antimicrobial activity of Lawsonia inermis Linn (henna). A pilot study on the Omani henna. *Saudi Med. J.* **2005**, *26* (1), 69–72.
14. Mikhaeil, B. R.; Badria, F. A.; Maatooq, G. T. Antioxidant and immunomodulatory constituents of henna leaves. *Z Naturforsch [C].* **2004**, *59* (7–8), 468–476.
15. Salunke-Gawali, S.; Rane, S. Y.; Puranik, V. G; Gugand-Duhayon, C.; Varret, f. Three dimensional hydrogen-bonding network in a copper complex of 2-hydroxy-1,4-naphthoquinone: Structural, spectroscopic and magnetic properties. *Polyhedron* **2004**, *23* (16), 2541–2547.
16. Bodini, M. E.; Bravo, P. E.; Aranciba, V. Voltammetric and spectroscopic study of the iron(II) complexes with the semiquinone of 2-hydroxy-1,4-naphthoquinone (lawsone) in aprotic medium. *Polyhedron* **1994**, *13* (3), 497–503.
17. El-Hendawy, A. M. Complexes of lawsone with uranium, molybdenum, ruthenium and osmium, and their use as organic oxidants. *Polyhedron* **1991**, *10* (20–21), 2511–2518.
18. Garge, P.; Padhye, S.; Tuchagues, J. Iron(II) complexes of ortho-functionalized para-naphthoquinones 1. Synthesis, characterization, electronic structure and magnetic properties. *Inorg. Chim. Acta.* **1989**, *157* (2), 239–249.
19. Christianopoulou, M. Synthesis and studies of some bivalent metal chelates of 1,2-dihydroxy-9,10-anthracenedione and 5-hydroxy-1,4-naphthalenedione. *Polyhedron* **1984**, *3* (6), 7–29.
20. Christianopoulou, M.; Ecateriniadou, L.; Sarris, E. Evaluation of the antimicrobial activity of a new series of hydroquinone chelates of some transition metals. *Eur. J. Med. Chem.* **1986**, *21* (5), 385–390.
21. Papageorgiou, V. P.; Christianopoulou, M.; Boutis, L.; Papageorgiou, A.; Tsipis, C. Synthesis and antitumor activity of a novel diplatinum complex of the binucleating naphthazarinato ligand. *Inorg. Chim. Acta* **1986**, *124* (4), 203–206.
22. Bakola-Christianopoulou, M.; krivos, P. D.; Ecateriniadou, L. B.; Sarris, K. On the anti-bacterial activity of non-charged μ-naphthazarinato compounds. *Eur. J. Med. Chem.* **1988**, *23* (1), 87–90.
23. Lialiaris, T.; Mourelatos, D.; Boutis, L.; Papageorgiou, A.; Christianopoulou, M.; Papageorgiou, V.; Dozi-Vassiliades, J. Comparative study on cytogenetic effects by diplatinum complexes of the ligands of naphthazarine and squaric acid in human lymphocytes. *J. Pharmacol. Exp. Therap.* **1989**, *251* (1), 368–371.
24. Gielen, M.; Willem, R.; Biesemans, R.; Boulam, M.; Khloufi, A.; Elde Vos, D. Exceptionally high in vitro antitumor activity of substituted triphenyltin benzoates including salicylates against a human mammary tumor, MCF-7, and a colon carcinoma, WiDr. *Appl. Organometal. Chem.* **1992**, *6* (3), 287–291.
25. Gielen, M. Tin-based antitumour drugs. *Coord Chem. Rev.* **1996**, *151*, 41–51.
26. Gielen, M.; Biesemans, M.; de Vos, D.; Willem, R. Synthesis, characterization and in vitro antitumor activity of di- and triorganotin derivatives of polyoxa- and biologically relevant carboxylic acids. *Inorg. Biochem.* **2000**, *79*, 139–145.
27. Gielen, M. Organotin compounds and their therapeutic potential: A report from the organometallic chemistry department of the free university of Brussels. *Appl. Organometal. Chem.* **2002**, *16*, 481–494.
28. Gielen, M.; Biesemans, M.; Willem, R. Organotin compounds: from kinetics to stereochemistry and antitumour activities. *Appl. Organometal. Chem.* **2005**, *19*, 440–450.
29. Pellerito, L.; Nagy, L. Organotin(IV)n+ complexes formed with biologically active ligands: equilibrium and structural studies, and some biological aspects. *Coord. Chem. Rev.* **2002**, *224*, 111–150.
30. Brown, M.A.; McGarvey, B. R.; Ozarowski, A.; Tuck, D. G. Studies of organotin(IV)-orthoquinone systems. *J. Organometal. Chem.* **1998**, *550* (1–2), 165–172.
31. Sisido, K.; Takeda, Y.; Kinugawa, Z. Direct synthesis of organotin compounds. I. Di and tri-benzyltin chlorides. *J. Am. Chem. Soc.* **1960**, *83*, 538–541.
32. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **1990**, *82* (13), 1107–1112.
33. Marchetti, F.; Pettinari, C.; Cingolani, A.; Pettinari, R.; Rossi, M.; Caruso, F. Organotin(IV) derivatives of novel β-diketones: Part V. Synthesis and characterization of di- and triorganotin(IV) derivatives of 4-acyl-5-pyrazolones modified in position 3 of the pyrazole. Crystal structure of (1,3-diphenyl-4-benzoyl-pyrazolon-5-ato)triphenyltin(IV). *J. Organomet. Chem.* **2002**, *645* (1–2), 134–145.

34. Mahon, M. F.; Molloy, K. C.; Omotowa, B. A.; Mesubi, M. A. Organotin(IV) derivatives of acylpyrazol-5-ones. *J. Organometal. Chem.* **1996**, *511*, 227–237.
35. Whiffen, D. H. Vibrational frequencies and thermodynamic properties of fluoro-, chloro-, bromo-, and iodo-benzene. *J. Chem. Soc.* **1956**, 1350–1355.
36. Ortiz, A.; Teruel, J. A.; Aranda, F. J. Effect of triorganotin compounds on membrane permeability. *Biochim. Biophys. Acta.* **2005**, *1720* (1–2), 137–142.
37. Sherman, L. Relationship of cytotoxic groups in organotin molecules and the effectiveness of the compounds against leukaemia. *Appl. Organometal. Chem.* **1988**, *2* (1), 65–72.
38. d'Arcy Doherty, M.; Rodgers, A.; Cohen, G. M. Mechanisms of toxicity of 2- and 5-hydroxy-1,4-naphthoquinone; absence of a role for redox cycling in the toxicity of 2-hydroxy-1,4-naphthoquinone to isolated hepatocytes. *J. Appl. Toxicol.* **1987**, *7* (2), 123–129.
39. Babich, A.; Stern, A. In vitro cytotoxicities of 1,4-naphthoquinone and hydroxylated 1,4-naphthoquinones to replicating cells. *J. Appl. Toxicol.* **1993**, *13*(5), 353–358.
40. Cooke, G. M. Effect of organotins on human aromatase activity in vitro. *Toxicol Lett.* **2002**, *126* (2), 121–130.
41. Osada, S.; Nishikawa, J.; Nakanishi, T.; Tanaka, K.; Nishihara, T. Some organotin compounds enhance histone acetyltransferase activity. *Toxicol. Lett.* **2005**, *155* (2), 329–335.
42. Bolton, J. L.; Pisha, E.; Zhang, F.; Qiu, S. Role of quinoids in estrogen carcinogenesis. *Chem. Res. Toxicol.* **1998**, *11* (10), 1113–1127.
43. Szorcsik, A.; Nagy, L.; Gajda-Schrantz, K. Structural studies on organotin (IV) complexes formed with ligands containing {S, N, O} donor atoms. *J. Radioanal. Nucl. Chem.* **2002**, *252* (3), 523–530.
44. Nath, L. M.; Pokharia, S.; Yadav, R. Organotin(IV) complexes of amino acids and peptides. *Coord. Chem. Rev.* **2001**, *215* (1), 99–149.
45. Gaussian 98; Revision, A.11. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E., Jr.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M.W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Gordon, M.; Replogle, E. S.; Pople, J. A.; Gaussian, Inc.; Pittsburgh, PA, 2001, Gaussian 98 program.