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Effect of diorganotin(IV) carboxylate complexes, [*N*-(2-carboxyphenyl) salicylideneiminato]dimethyltin(IV), bis(μ_3-oxo)bis(μ-*O*-aminobenzoato-*O*,*O*′) bis(*O*-aminobenzoato)tetrakis[dimethyltin(IV)] and bis(*O*-aminobenzoato-*O*,*O*′) di-*n*-butyltin(IV), on the membrane of *Candida albicans* cells – a mechanistic investigation of the antifungal activity of organotin complexes

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The *in vitro* anti-fungal activity of diorganotin(V) carboxylate complexes, [*N*-(2-carboxyphenyl)salicylideneiminato] dimethyltin(IV) (1), bis(μ_3-oxo)bis(μ-*O*-aminobenzoato-*O*,*O*′)bis(*O*-aminobenzoato)tetrakis[dimethyltin(IV)] (2) and bis(*O*-aminobenzoato-*O*,*O*′)di-*n*-butyltin(IV) (3) has been screened against *Candida albicans*. The complexes, prepared by minor modifications of a literature method, have been fully characterized. The biological activities of complexes 1–3 on cultures of *Candida albicans* have been studied and the related mechanism has been investigated. No changes in DNA integrity or in the mitochondria function have been observed. However, all the tin compounds were found to reduce the ergosterol biosynthesis. The compounds have inhibitory concentrations (IC_{50}) in the range of 7.5–20.0 μg ml^{-1}, with compound 3 the most active. These results were compared with those provided by the literature, using myconazole, amphotericin B and fluconazole as standards. Scanning electron microscopy and transmission electron microscopy of the cells treated with complexes 1–3, revealed severe damage on the *C. albicans* cells compromising the cellular integrity, suggesting that the organotin complexes act on the cell membrane, in view of cytoplasm leaking and cellular deformation. The data indicate for 1–3 a mechanism of action similar to that of azole drugs, such as ketoconazole or fluconazole, normally used in *Candida* infections.

Keywords: organotin compounds; *Candida albicans*; antifungal activity

Introduction

Despite some fungi infections being difficult to treat in humans and other mammals, some fungi species are useful in the research of new anti-neoplastic drugs, providing invaluable insights to the mechanism of their action.[1] *S. cerevisiae* has indeed been used as a test for new chemotherapeutic agents.[2,3] Many compounds which display antifungal activity have also been found to have an anticancer effect.[3,4]

The range of potential biological applications of organotin compounds has been widely reported.[5] Anti-oxidant,[6] anti-inflammatory and vasodepressor[7] activities are among those described in the literature.[8] Other activities include (i) schizonticidal and antimalarial activities exhibited by aminoquinoline-tin complexes;[9] (ii) and amoebicidal activity of tin-Schiff base complexes.[10] Tin-2-alkylindole derivatives have been tested against *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus* and

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Micrococcus luteus[11] and dioctyltin maleate has been found active against *leshmaniasis* in mice and *helminthes* in cats. One of the more interesting biological activities of organotin(IV) complexes is their antitumor activity.[12–14]

The interaction of organotin derivatives with biologic systems has been well documented.[15,16] A number of mechanisms for the biological action of organotin derivatives have been proposed.[17] However, a complete understanding has still to be found. Release of K^+ from cells, resulting from increased cytoplasmic membrane permeability points out the cytoplasmic membrane as possible site of action.[18–20] The crossing of the cytoplasmatic membrane by organotin derivatives might be a consequence of lipid-solubility[21,22] effected by weak interactions involving amino-acids, proteins,[23] nucleosides, carbohydrates and steroids,[24] or by anionic carboxylates, hydroxyl and phosphate groups present in the cell membrane binding cationic organotin fragments at the cell surface.[25] Nevertheless, the effectiveness of the biologic activity of organotin derivatives depends on the structure.[26] The toxicity of organotin compounds has been found to be a function of the number, as well as the nature, of the organic groups attached to the metal centre. Normally tri-organotin compounds have higher biocidal actions than di-organotin species, although the latter have good antitumor activity. On the other hand, mono-organotin compounds possess little effect on biologic systems. Some authors have proposed that toxicity in the R_3Sn series correlates with total molecule surface (TSA) and hence *n*-propyl-, *n*-butyl-, *n*-pentyl-, phenyl- and cyclohexyl-substituted tin compounds should be more toxic than ethyl- and methyl-tin, supporting a toxicity and lipophilicity correlation.[25]

In this paper, we report some results involving organotin carboxylic derivatives (previously screened for their antitumor activity), [*N*-(2-carboxyphenyl)salicylideneiminato]dimethyltin(IV) (**1**), bis(μ_3-oxo)bis(μ-*O*-aminobenzoato-*O*,*O*′)bis(*O*-aminobenzoato) tetrakis[dimethyltin(IV)] (**2**) and bis(*O*-aminobenzoato-*O*,*O*′)di-*n*-butyltin(IV) (**3**), and cultures of *C. albicans*. We have (i) determined the inhibitory concentration (IC_{50}) for compounds **1–3**; (ii) examined the integrity of the genetic material and respiratory functions of drug-treated colonies of *C. albicans*; (iii) measured the levels of ergosterol in the cell membrane; and (iv) observed the morphology of the cells through scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Compound **3** has been screened and showed remarkable *in vitro* antitumor activity against the human tumor cell lines, MCF-7 (mammary tumor) and WiDr (colon carcinoma).[27]

Materials and Methods

The diorganotin (IV) carboxylates investigated in this study, [*N*-(2-carboxyphenyl)salicylideneiminato]dimethyltin(IV) (**1**), bis(μ_3-oxo)bis(μ-*O*-aminobenzoato-*O*,*O*′)bis(*O*-aminobenzoato)tetrakis [dimethyltin(IV)] (**2**) and bis(*O*-aminobenzoato-*O*,*O*′)di-n-butyltin(IV) (**3**), Fig. 1, have been prepared and characterized according to the literature procedure with minor modifications.[28–30] The ligand (2-$HOC_6H_4CH{=}NC_6H_4COOH$) was prepared according to a literature procedure.[23] Complex (**1**) was obtained by reacting 2-$HOC_6H_4CH{=}NC_6H_4COOH$ with $SnMe_2Cl_2$, in methanol, in the presence of Et_3N. The other complexes were prepared in a similar manner using 2-aminobenzoic acid and the appropriate organotin derivative $SnMe_2Cl_2$ (**2**) or $SnBu_2Cl_2$ (**3**). All compounds were authenticated using appropriate techniques. Elemental analyses were performed using a Perkin-Elmer Model PE 2400CHN. Melting points were determined with a digital melting point from Mettler model FP90 with cell of heating model FP82 HT and microscopy from Olympus CH-2. IR spectra were obtained as KBr plates on a Mattson Galaxy model ST 3000 spectrometer in the 4000–200 cm^{-1} range. NMR spectra were recorded in $CDCl_3$ at 25 °C on a Brucker Avance DRX 400. The chemical shift values were referenced to internal $SiMe_4$ and $SnMe_4$. ^{119}Sn Mössbauer measurements were performed using a conventional apparatus with the samples at liquid N_2 temperature and a $CaSnO_3$ source kept at room temperature. We have also reviewed the structural determination of compound **2** and the structure has been deposited at CCDC (reference no. 650424).

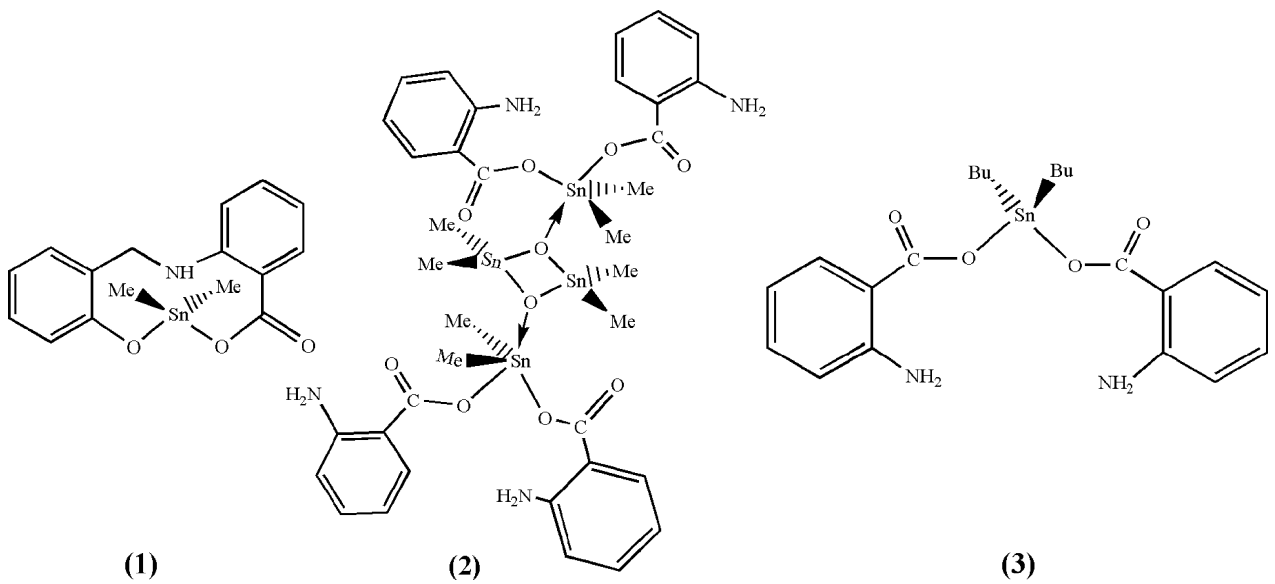

Figure 1. Structures of the compounds: [*N*-(2-carboxyphenyl)salicylideneiminato]dimethyltin(IV) (**1**), bis(μ_3-oxo)bis(μ-*O*-aminobenzoato-*O*,*O*′)bis(*O*-aminobenzoato)tetrakis[dimethyltin(IV)] (**2**) and bis(*O*-aminobenzoato-*O*,*O*′)di-*n*-butyltin(IV) (**3**).

[N-(2-carboxyphenyl)salicylideneiminato]dimethyltin(IV) (**1**)

Yield 55%; m.p. 294.4–295.6 °C. IR (CsI, cm^{-1}): 1660 w (ν_{COO}); 1604 s ($\nu_{C=N}$); 222 w (ν_{SnO}). ^1H NMR (DMSO-*d6*, 400.13 MHz), δ 8.8 s (1H, CH=N), 8.0–6.7 m (8H, aromatic), 0.5 (6H, s, $\{CH_3\}_2Sn$). $^{13}C\{^1H\}$ NMR (DMSO-d6, 100.61 MHz), δ 199.8 (COO); 170.2 (CH=N); 151.1–114.1 m (12 C, aromatic); 7.3 $\{CH_3\}_2Sn$. $^{119}Sn\{^1H\}$ NMR (DMSO-d6, 149.21 MHz), δ −328. ^{119}Sn-Mössbauer (mm s^{-1}), δ 1.29, Δ 3.76. Analysis for $C_{16}H_{15}NO_3Sn$, found (calcd): C 49.63 (49.53), H 3.70 (3.89), N 3.69 (3.61).

bis(μ_3-oxo)bis(μ-O-aminobenzoato-O,O′)bis(O-aminobenzoato)tetrakis[dimethyltin(IV)] (**2**)

Yield 65%; m.p. 238.0–240.0 °C. IR (CsI, cm^{-1}): 1616 s (ν_{COO}); 1366 s ($\nu_{s\ COO}$); 650 w (ν_{SnO}). ^1H NMR ($CDCl_3$, 400.13 MHz), δ 7.8–6.7 m (4H, aromatic), 1.0 s (CH_3), 5.7 s (NH_2). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100.61 MHz), δ 175.1 (COO); 150.4–113.1 m (6C, aromatic); 7.3 (CH_3). $^{119}Sn\{^1H\}$ NMR ($CDCl_3$, 149.21 MHz), δ−190.5 and −172.9. ^{119}Sn-Mössbauer (mm s^{-1}), δ 1.51, Δ 3.72 and δ 1.37, Δ1.29. Analysis for $C_{36}H_{46}N_4O_{10}Sn_4$, found (calcd): C 46.48 (46.28), H 5.09 (5.17), N 5.93 (5.99).

bis(O-aminobenzoato-O,O′)di-n-butyltin(IV) (**3**)

Yield 80%; m.p. 114.4–116.0 °C. IR (CsI, cm^{-1}): 1623 s (ν_{COO}); 1366 s ($\nu_{s\ COO}$); 671 w (ν_{SnO}). ^1H NMR ($CDCl_3$, 400.13 MHz), δ 8.0–6.7 m (4H, aromatic ring), 5.7 s (NH_2), 1.7–0.8 (C_4H_9). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100.61 MHz), δ 177.3 (COO); 150.3–111.0 m (6C, aromatic); 26.7 (CH_2-α); 26.3 (CH_2-β); 25.5 (CH_2-γ); 13.5 (CH_3). $^{119}Sn\{^1H\}$ NMR ($CDCl_3$, 149.21 MHz), δ−151.3. ^{119}Sn-Mössbauer (mm s^{-1}), δ 1.20, Δ 3.62. Analysis for $C_{22}H_{30}N_2O_4Sn$, found (calcd): C 52.40 (52.30), H 6.04 (5.98), N 5.59 (5.54).

Chemicals for the biological tests

2,3,5-Triphenyltetrazolium chloride, agarose, ethidium bromide, potassium hydroxide, solvents and reagents for the preparation of cultures were obtained from Aldrich, Merck and Synth and used as received. The chemicals for culture media, agar and dextrose broth Sabouraud were obtained from Biobrás S.A., MG, Brazil. Poly-L-lysine and carbon dioxide were purchased from Sigma and Balzer, respectively.

Yeast strain and culture conditions

Yeast cells of *Candida albicans*, ATCC 18804, were seeded and grown aerobically in Sabouraud dextrose agar (SDA) at 37 °C for 24 h and stored at 4 °C. Suspensions were prepared in concentration range of 10^5–10^6 CFU ml^{-1} (determined by the McFarland scale, CFU = colony-forming unit) in Sabouraud dextrose broth (SDB) for IC experiments, and grown at 37 °C for 24 h. For yeast cell viability, DNA integrity assays and lipid extractions *C. albicans* cultures were grown on stationary phases at 30 °C (10^9 CFU ml^{-1}, determined by Neubauer cell apparatus) with continuous shaking in SDB. The complexes were dissolved in DMSO and all experiments were carried out in triplicate.

Inhibitory concentrations

Inhibitory concentration (IC) experiments were performed with minor modifications of the method described in the literature.[31] IC_{50} and IC_{95} values were defined as the minimum concentration of drug required to inhibit the growth of 50 and 95% of the fungal culture at 37 °C. An aliquot of 10 µl of DMSO was mixed with the culture medium as the control. After 24 h of incubation, data were collected by spectroscopic analysis (absorbance at λ = 600 nm).

DNA integrity assays from *C. albicans*

This assay was performed in order to observe any possible cellular apoptosis effect by DNA fragmentation. We based our strategy on a method used in previous studies.[32] *C. albicans* cells were grown in SDB medium at 30 °C, with shaking, for 24 h in two different conditions: (i) the presence of 10 µl DMSO (control sample); (ii) the presence of each complex at a concentration of 40 µg ml^{-1}. The liquid cultures were centrifuged for 5 min. The liquid phase was eliminated and pellets were re-suspended in 200 µl lysis buffer (2% Triton X-100; 1% sodium dodecylsulfate; 100 mmol l^{-1} NaCl; 10 mmol l^{-1} Tris–HCl pH = 8; 1 mmol l^{-1} EDTA pH = 8). The tubes were then immersed in liquid nitrogen for 2 min (twice) and transferred to a hot bath at 95 °C for 1 min. The tubes were vortexed for 30 s. Then, 200 µl of chloroform were added and the tube was agitated for 2 min. Then they were centrifuged again for 3 min and the aqueous phase was transferred to tubes containing 400 µl of cold ethanol. The tubes were incubated overnight at −20 °C, centrifuged and the pellets washed with 500 µl cold ethanol (70%) and dried at room temperature. Finally, the pellets were re-suspended in 10 µl TE buffer (10 mmol l^{-1} Tris–HCl pH = 8 and 1 mmol l^{-1} EDTA pH = 8). Alterations in DNA integrity were assessed by comparing electrophoretic migration of the samples in a 0.8% agarose gel prepared in TAE buffer [40 mmol l^{-1} tris(hydroxymethyl)aminomethane acetate and 1 mmol l^{-1} EDTA]. For each assay 5 µl of the TE suspensions were mixed with 5 µl of loading buffer (0.025 mg bromophenol blue; 1 ml glycerol and 1ml distilled water) and transferred to wells in the horizontal gel. A potential difference of 70 mV was applied over the gel for approximately 0.5 h and the bands were stained with ethidium bromide and visualized under UV light.

Yeast cell respiratory viability

A respiratory test was carried out to detect any drug-induced change in the respiratory function of the fungus.[33,34] Cultures of *C. albicans* were grown for 24 h at 37 °C in the presence of the organotin compounds at concentrations of 10 and 40 µg ml^{-1}. Then, the mixture was centrifuged at 1.3×10^4 rpm for 5 min and the cells were suspended in phosphate buffered saline, PBS (NaCl, 137 mmol l^{-1}; KCl, 2.7 mmol l^{-1}; Na_2HPO_4, 10 mmol l^{-1}; KH_2PO_4, 2 mmol l^{-1}; pH = 7.4). Each cell suspension (100 µl) was spread over 15 ml of SDA on Petri dishes and incubated for 48 h at 30 °C. Then the samples were transferred to Petri dishes with molten agar (2% agar, 0.5% glucose and 2,3,5-triphenyltetrazolium chloride, TTC, 0.05% w/v) and grown for 24 h at 30 °C. The number of colonies was ascertained and expressed as a percentage with respect to the control (100%) and the results were statistically analysed by non-paired *t*-test with significance level, $p > 0.05$. Colonies undergoing active respiration reduce TTC to a dark red color while those with reduced respiratory function remain white.

Ergosterol extraction

Ergosterol and dehydroergosterol were extracted employing a methodology described in the literature with minor

modifications.[35] Briefly, the organotin compounds (at concentration of 40 μg ml^{-1}) were added to cells grown on 2 ml of SDB with continuous shaking at 30 °C for 24 h. A suspension of the culture containing DMSO was used as control. The liquid cultures were transferred to Eppendorf tubes and centrifuged for 5 min at 1.3×10^4 rpm. The pellets were washed with distilled water, dried at room temperature and weighed. The resulting cells were re-suspended in 25% alcoholic potassium hydroxide solution (25 g of KOH in 35 ml of sterile distilled water and 65 ml of ethanol, giving a final cell density of 1×10^9 cells cm^{-3}), incubated at 90 °C for 1 h, and cooled to room temperature. The lipids were extracted by adding 0.5 ml sterile distilled water and 1 ml of *n*-heptane, and vortexed for 3 min. The *n*-heptane layer was transferred to Eppendorf tubes and kept at −20 °C for 24 h. Aliquots of 2 μl were diluted in ethanol and analysed ($\lambda = 230$ and 281.5 nm). The concentration of lipids was calculated according to the published methodology[35] by measuring the difference in the absorbance values at 281.5 and 230 nm. Finally, the results were statistically analysed by *t*-test (non-paired Student's test) with significance level, $p > 0.05$.

SEM and TEM evaluation

Cultures of *C. albicans* were grown for 24 h at 37 °C, in the presence of each compound, at concentrations of 20.0, 17.5 and 7.5 μg ml^{-1} to complexes **1–3** (IC_{50}) respectively. Compound **3** was also employed at a concentration of 20 μg ml^{-1} (95% of inhibition). Cultures grown in the absence of the compounds were used as control. The cultures were centrifuged at 3×10^3 rpm for 1 min. The primary fixation procedure of stationary phase yeast cells was in 2.5% glutaraldehyde buffered with 0.1 mol l^{-1} sodium cacodylate, pH 7.2, at 4 °C for 3 h. The resulting solid was rinsed with cacodylate buffer, centrifuged and the samples allocated to TEM analysis were mixed with agar (3%). For TEM experiments the samples were post-fixed in potassium ferrocyanide-reduced 1% osmium tetroxide in the same buffer for 30 min at 4 °C. Dehydration of samples was achieved in acetone (30, 50, 70, 90 and 100%), each one for 10 min. The samples were infiltrated (1 : 2; 1 : 1; 2 : 1) in epon (resin : acetone). The polymerization process occurred after 72 h at 65 °C. Semi-thin sections were stained with toluidine blue and ultra-thin sections about 80–90 nm thick were double-stained with uranyl acetate and lead citrate; the samples were cut using an Ultracut E–Sorvall apparatus with diamond blades. The specimens were examined in a Zeiss-EM 10 transmission electron microscope. For the SEM analysis, small drops of the samples were placed on a specimen support with poly-L-lysine. Post-fixation was carried out with 1% of osmium tetroxide in cacodylate buffer for 30 min, with 1% of tannic acid. Subsequently, the specimens were dehydrated in graded ethanol solution in which the concentration was increased from 30% (30, 50, 70, 80, 90, 95%) for 30 min and 100% for two periods of 15 min. Then, it was dried using liquid carbon dioxide (critical), and coated with a thin gold film and analysed in a scanning electron microscope (Zeiss-DSM 950).

Results and Discussion

The reaction between carboxylic acids with the appropriate organotin chloride in the presence of NEt_3 yielded the complexes **1–3**, which were fully characterized.

Inhibitory concentrations (IC_{50}) of [N-(2-carboxyphenyl) salicylideneiminato]dimethyltin(IV) (1), bis(μ_3-oxo)bis (μ-O-aminobenzoato-O,O′)bis(O-aminobenzoato)tetrakis [dimethyltin(IV)] (2) and bis(O-aminobenzoato-O,O′) di-n-butyltin(IV) (3)

The inhibitory concentrations, IC_{50}, for the compounds were 20.0 μg ml^{-1} (**1**), 17.5 μg ml^{-1} (**2**) and 7.5 μg ml^{-1} (**3**), and the IC_{95} for compound **3** was 20.0 μg ml^{-1} and can be compared with the IC_{50} values of the clinical agents in use, myconazole, amphotericin B and fluconazole, 110.8, 1.00 and 8.00 μg ml^{-1}, respectively.

DNA integrity assay

A well-known literature method used previously to indicate DNA fragmentation of cells exposed to Cu(II), Mn(II) and Ag(I) 1,10-phenanthroline complexes was employed.[32] In our work, DNA was extracted from the control cell and cultures exposed to **1, 2** and **3**, each at a concentration of 40 μg ml^{-1}. Little difference was observed between the results of the control and test cultures for each of the tin complexes. Degradation of high molecular weight DNA was not evident. Either the organotin concentrations were not sufficient to induce fungal cell DNA degradation, or the cells were capable of repairing the damage over the timeframe of the experiment. However the concentrations were higher than that corresponding to IC_{50}. Our results provide no evidence for the interaction between organotin complexes and DNA.

Yeast cell respiratory viability

C. albicans colonies display colors ranging from yellow to off-white.[36] During the respiration process, glucose is consumed, resulting in the reduction of 2,3,5-triphenyltetrazolium chloride (TTC), causing a color change to red. No color change is observed when external agents compromise the respiratory function, since the cells lose the ability to promote the chemical reduction.[33,37] Studies have revealed that some organotin derivatives interact with mitochondria, inducing cytochrome c release by a mechanism inhibited by cyclosporine A and bondkrekic acid.[15c] It was expected that complexes **1–3** would provide similar results.

After 24 h of exposure to **1, 2** or **3**, at concentrations of 10 and 40 μg ml^{-1}, *C. albicans* colonies were counted and expressed as a percentage with respect to the control (100%). Color changes from white to red were detected, suggesting that most of the drug-treated cells, at fungicidal concentration of complexes, preserve the respiratory function. It has been reported that that $[Mn(phen)_2(mal).2H_2O]$, $[Ag_2(phen)_3(mal).2H_2O]$, $[Cu(phen)_2(mal).2H_2O]$, cisplatin and palladium complexes cause a significant alteration in oxygen uptake in *C. albicans* cells.[32,37] In the present study minimal changes were observed in the respiratory function, indicating that the antifungal activity of the organotin derivatives is not related to it.

Ergosterol content

The relative ergosterol contents of drug-treated and control cells were determined (Fig. 2). All three organotin derivatives (40 μg ml^{-1}) decreased the ergosterol levels in the cell membrane, compared with that of the untreated control group – a suspension of the culture in DMSO. Compounds **1** and **2** caused reductions of about 35 and 44%, respectively, and compound **3** 76%. This is evidence of the interference of the tested compounds with the biosynthesis of lipids.

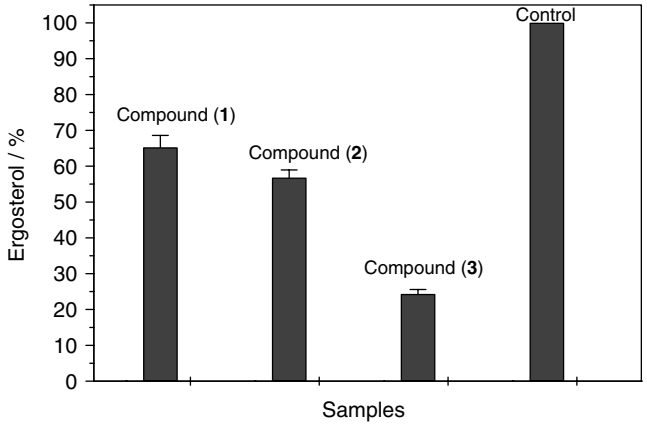

Figure 2. Relative ergosterol contents of compound-treated and control cells.

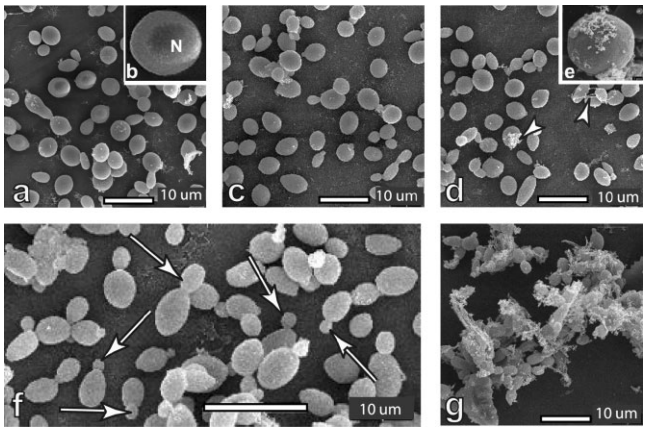

Figure 3. Scanning electron microscopic images: (a) control culture; (b) projection of the nucleus on the cell surface; (c) cells treated with compound (**1**); (d) cells treated with compound (**2**); (e) detail of the deposition of residues on the surface of cells treated with compound (**2**); (f) cells treated with compound **3** at IC_{50}. Observe cell budding (arrows). (g) Cells treated with compound **3** at IC_{95}.

The literature describes ergosterol reduction of 38, 57, 73, and 99% after exposure of *C. albicans* cells to 1, 4, 16 and 64 μg of fluconazole ml^{-1} respectively.[35] Voriconazole treatment led to a decrease in the total sterol content of fluconazole-resistant *C. albicans* strains.[38] Our best result was displayed by complex **3**, but, this was not as good as that of fluconazole or voriconazole.

Electron microscopy results (SEM and TEM)

SEM and TEM experiments were carried out in order to investigate the morphology of *C. albicans* cells previously exposed to compounds **1–3**. Samples of *C. albicans* exposed to complexes **1–3** at concentrations corresponding to their IC_{50} values were employed (Fig. 3). An additional SEM experiment was performed using compound **3** at the IC_{95} concentration [Fig. 3(g)].

Normal aspects were observed for the cells in the control samples. The SEM images revealed normal egg-shaped morphologies, uniform surfaces and projections of regular and large nucleus on the surfaces of some cells[36] [Fig. 3(a, b)]. Colonies subjected to compounds **1** and **2** present morphologies similar to those of the control group [Fig. 3(c–e)]. However, the presence of residues, either free or connected to a minor number of cells treated with

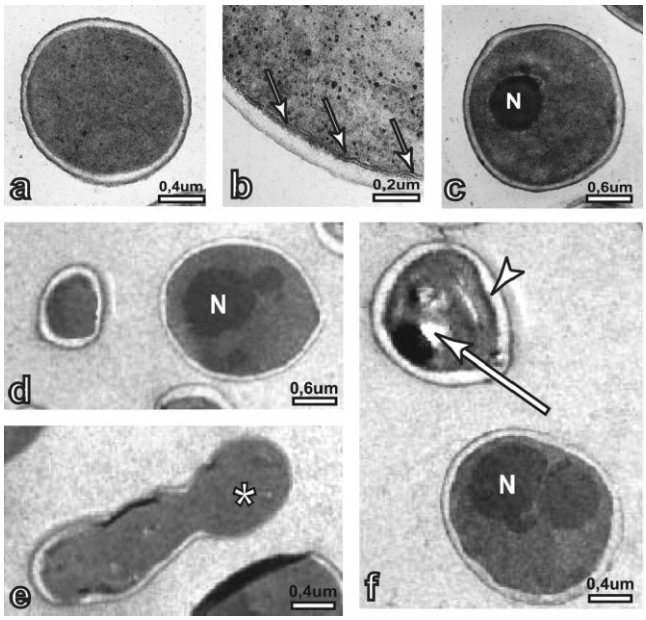

Figure 4. Transmission electron microscopic images obtained. Control: (a) *C. albicans* in control culture; (b) cell wall and double plasmatic membrane; (c) control cell with evident nucleus. Cells exposed to compound **1** at IC_{50}: (d) shapeless and irregular nuclear material; (e, f) cells with irregular membrane.

2, indicates minor cellular leakage [Fig. 3(d)]. Cells treated with **3** presented more budding, compared with control sample and also with those submitted to **1** and **2** [Fig. 3(f)]. In addition the SEM experiments suggest that **1** and **2** do not affect greatly the proliferation and cellular growth. The sample subjected to **3** at the IC_{95} showed a high level of cellular damage [Fig. 3(g)]. The SEM experiments failed to show details of the internal structure of the cell after contact with the organotin compounds. Therefore, we performed TEM experiments in order to visualize internal cell damage. Ultra-thin sections of control cells displayed normal structure in terms of cytoplasm, cellular membrane and nucleus [Fig. 4(a–c)]. The TEM results revealed colonies at the toxicity threshold with significant intracellular alterations. The TEM images of the *C. albicans* cultures treated with compound **1** showed that most of the cells were preserved. However cells were observed with cytoplasm leaking and irregular nuclear material [Fig. 4(e–f)]. The inner aspects of the cells exposed to **2** is not very different from those treated with **1** [Fig. 5(a–c)]. On the other hand, **3** produced strong deterioration in the cellular membrane, as pointed out by the SEM images and the ergosterol tests [Fig. 5(d–f)].

The antifungal activities of azole derivatives such as miconazole, clotrimazole, bifonazole, ketoconazole, itraconazole, fluconazole and voriconazole arise from a complex multimechanistic process initiated by the inhibition of a cytochrome P450 (CYP) involved in the biosynthesis of ergosterol, namely, enzyme sterol-14α-demethyllase (CYP51).[39]

Amphotericin and nystatin, classical examples of polyene drugs for *Candida* infections, act on the membrane of the fungi cells by linking to ergosterol. These drugs interfere with the permeability of the cellular membrane, causing losses of macromolecules and ions essential for cell survival.[40,41] Interaction with CYP51 results in a decreased mitochondrial function and availability of ergosterol and accumulation of 14-methylsterols. Changes in ergosterol levels

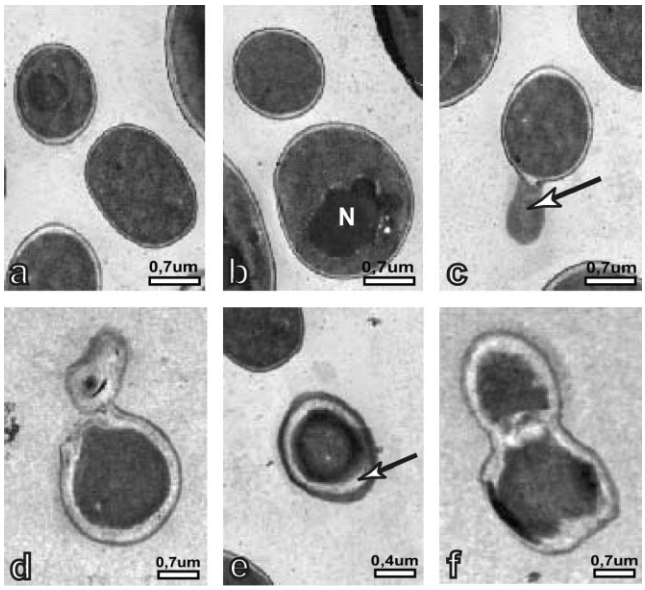

Figure 5. Transmission electron microscopic images obtained at IC_{50}. Cells treated with compound (**2**): (a) cell with normal aspect; (b) irregular nucleus; (c) cell with leaking of cytoplasm content. Cells treated with compound **3**: (e) cell with leaking of cytoplasm content; (d, f) cells with irregular membrane.

and sterol structures influence the activity of several metabolic pathways and also membrane permeability.[42]

All three organotin compounds examined in this work show antifungal activity with the di-butyltin derivative, **3**, being the most active. Our results agree with those found in the literature for similar species, which show for butyl-containing species higher biologic activity than the methyl analogs. We believe that the antifungal activity of complex **3** relates to a higher molecular surface (TSA) of the R_2Sn fragment,[25] resulting in greater lipophilic interaction with cellular membranes. This activity is not related to the respiratory viability, as observed for complexes of other metals or even for some organotin derivatives.[15c] No mitochondrial changes were detected in this work. The interaction of organotin derivatives with lipids is documented in the literature;[17] therefore, the interference of complexes **1**–**3** with the ergosterol biosynthesis is not an unexpected result. The DNA assay indicates that **1**–**3** did not affect cellular integrity at the level of genetic material. Morphological studies performed using SEM and TEM techniques confirmed the results, revealing that the organotin complexes induce damage of the cellular membrane and modify internal aspects of the cells. This is in agreement with the results obtained by the ergosterol content analyses, which revealed that all three complexes inhibit the biosynthesis of lipids, which is important for the integrity of the cell membrane.

Finally, we conclude that the actions of the organotin complexes are apparently similar to those of azole drugs such as fluconazole and ketoconazole. Therefore, the organotin complexes **1**–**3** may represent a new class of drugs to be employed alone or in new formulations with others in current use to overcome resistance of *C. albicans* isolates. The close relationship between antifungal and antitumor activity highlighted in a recent publication[1] add a greater significance to the present study, as it is anticipated that investigations of antifungal activities can lead to a better understanding of anti-neoplastic action, as shown, for example, by compound **3**.

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