Organotin(IV) Esters of 4-Maleimido-benzoic Acid: Synthesis, Characterization and in vitro Anti-leishmanial Effects

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Seis novos ésteres diorganoestanho(IV) com composição geral \( \text{R}_2\text{SnL}_2 \) (onde \( \text{R}: \text{Me}, \text{Et}, \text{Pr}, \text{Bu}, \text{Ph}, \text{Bz} \)) foram sintetizados neste trabalho. Espectros de absorção no infravermelho e de Mössbauer de \( ^{119m}\text{Sn} \) no estado sólido revelaram o comportamento bidentado de \( \text{L} \) em relação ao centro diorganoestanho(IV) nos complexos octaédricos distorcidos. Espectros de RMN de \( ^1\text{H}, ^{13}\text{C} \) e \( ^{119}\text{Sn} \), em CDCl\(_3\), indicaram hexacoordenação em \( 1-4 \), pentacoordenação de \( 5 \) em geometria trapezoidal distorcida, e ausência de hipercoordenação no arranjo tetraédrico em \( 6 \). Dados de análise elementar comprovaram a estequiometria dos compostos organoestanho(IV). Foram realizados testes in vitro contra cinco espécies de Leishmania: \( L. \text{major} \), \( L. \text{tropica} \), \( L. \text{infantum} \), \( L. \text{mex. mex.} \) e \( L. \text{donovani} \). Resultados promissores foram observados e, com base nos dados obtidos nesses ensaios, tentou-se estabelecer relações estrutura-atividade. O aumento no tamanho dos grupos \( \text{R} \) em \( \{\text{R}_2\text{Sn}^{IV}\}^{2+} \) aumentou a lipofilicidade dos complexos organoestanho(IV), acentuando assim a atividade antileishmaníase.

Six new diorganotin(IV) esters with the general composition \( \text{R}_2\text{SnL}_2 \) (where \( \text{R}: \text{Me}, \text{Et}, \text{Pr}, \text{Bu}, \text{Ph}, \text{Bz} \)) have been synthesized. Solid state FTIR and \( ^{119m}\text{Sn} \) Mössbauer spectra revealed bidentate behavior of \( \text{L} \) towards the diorganotin(IV) centre in the distorted octahedral products. \( ^1\text{H}, ^{13}\text{C} \) and \( ^{119}\text{Sn} \) NMR spectra in CDCl\(_3\), indicated hexacoordination in \( 1-4 \), penta-coordination of \( 5 \) in skew-trapezoidal geometry, and absence of hypercoordination in tetrahedral \( 6 \). Elemental analyses data have been found to corroborate the stoichiometry of the title organotin(IV) compounds. In vitro anti-leishmanial screenings have been conducted on five leishmanial strains of \( L. \text{major} \), \( L. \text{tropica} \), \( L. \text{infantum} \), \( L. \text{mex. mex.} \) and \( L. \text{donovani} \). Promising results have been observed and, on the basis of the data obtained during these assays, a structure-activity relationship has been suggested. The increasing size of the \( \text{R} \) groups in the \( \{\text{R}_2\text{Sn}^{IV}\}^{2+} \) moieties increased the lipophilicity of organotin(IV) complexes, which thereby enhanced the anti-leishmanial activity.

Keywords: organotin(IV), anti-leishmanial, SAR

Abbreviations

Me(methyl), Et(ethyl), Pr(n-propyl), Bu(n-butyl), Ph(phenyl), Bz(benzyl).

Introduction

Amino acids and their organic as well as organometallic derivatives present a wide range of noteworthy pharmacological applications. Transition metal complexes of \( N \)-protected amino acids are active against different types of microbes, but literature reveals that the coordinating ability of \( N \)-protected amino acids as ligands decrease the biological activity of their transition metal complexes up to a certain extent. Organotin(IV) compounds are well-known for their manifold implications, such as tumouricidal, bactericidal and fungicidal activities, and for their interesting structural features. Leishmaniasis is a parasitic disease in tropical countries and the number of leishmanial cases has increased alarmingly during the last decade. Triphenyltin(IV) complexes of salicylanilide thiosemicarbazone have been reported to be effective in vitro...
and in vivo as anti-leishmanial agents against L. donovani, and considered a good prospect as a therapeutic mediator for leishmaniasis. Keeping in view all these facts and our recent work, dealing with the synthesis of new cytotoxic organotin(IV) complexes utilizing biologically active molecules as ligands, in this communication we describe the synthesis and spectroscopic analyses of six new diorganotin(IV)-di-4-maleimido-benzoates, which have been screened in vitro for anti-leishmanial activity on five different leishmanial strains.

**Experimental**

**General**

Analytical Reagent (AR) grade chemicals used during this work were procured from Sigma or Fluka and used without purification. Dibenzyltin(IV) dichloride was prepared according to a reported procedure, and solvents were dried as reported.

**Instrumentation**

Elemental analyses (C, H, N) were performed on a Yanaco high-speed CHN analyzer with antipyrene as a reference, while tin content was estimated according to a reported procedure. Uncorrected melting points were taken on a Reichert Thermovar of F. G. Bode Co., Austria. The FTIR spectra of the p-N-maleimido-benzoic acid (L) and the complexes were measured on a Brüker FTIR TENSOR27 spectrophotometer using OPUS software in the range of 5000-500 cm⁻¹ (ZnSe). For Mössbauer measurements, the solid samples were maintained at liquid nitrogen temperature (77.3 K), and the equipment employed was a V.G. Micromass 7070 F Cryolid liquid nitrogen cryostat. The multichannel calibration was performed with an enriched iron foil using a ⁵⁷Co-Pd source, while the zero point of the Doppler velocity scale was determined through an enriched iron foil using a ⁵⁷Co-Pd source.

**Synthesis of organotin(IV) complexes**

A solution of the triethylammonium salt of 4-maleimido-benzoic acid (0.5 g, 0.0015 mol L⁻¹) in dry toluene (100 mL) was prepared and an appropriate amount of diorganotin(IV) dichloride (0.0008 mol L⁻¹) was added. This mixture was heated to reflux for 3 hours, resulting in the formation of triethylammonium hydrochloride, which was filtered off. The filtrate was evaporated on a rotary evaporator and the solid mass was triturated in n-hexane, dissolved in C₆H₆ and finally recrystallized from CH₂Cl₂.

**Spectral data for compounds (1-7)**

**Bis(4-maleimido-benzoato)dimethyltin(IV) (I)**

White solid, mp 158 °C. Yield: 81%. IR ν [cm⁻¹]: 1631 ν(COO)₁, 1447 ν(COO)₂, Δν: 184, 411 ν(Sn–O), 522 ν(Sn–C)₁, 517 ν(Sn–C)₂, 119mSn Mössbauer (mm s⁻¹): QS: 3.32, IS: 1.32, Γ₁: 0.98, Γ₂: 0.87, ρ: 2.50. ¹H NMR (CDCl₃) δ: 7.8 (d, J = 7.1 Hz, 1H, CH), 7.7 (d, J = 7.1 Hz, 1H, CH), 7.1 (d, J = 7.3 Hz, 1H, CH), 0.6 (s, 3H, CH₃); ¹³C NMR (75 MHz, CDCl₃) δ: 175.4(1C), 133.6(2C), 136.8(3C); ¹¹⁹Sn Mössbauer (mm s⁻¹): QS: 3.32; IS: 1.32; Γ: 0.98; ρ: 2.50. ¹H NMR (CDCl₃) δ: 7.8 (d, J = 7.1 Hz, 1H, CH), 7.7 (d, J = 7.1 Hz, 1H, CH), 7.1 (d, J = 7.3 Hz, 1H, CH), 0.6 (s, 3H, CH₃); ¹³C NMR (75 MHz, CDCl₃) δ: 175.4(1C), 133.6(2C), 136.8(3C); ¹¹⁹Sn Mössbauer (mm s⁻¹): QS: 3.32; IS: 1.32; Γ: 0.98; ρ: 2.50.
C26H22N2O8Sn: C, 51.46; H, 3.64; N, 4.60; Sn, 19.19. Found: Bis(4-maleimido-benzoato)dibutyltin(IV) (18.63. Found: C, 52.70; H, 4.02; N, 4.01; Sn, 18.45. Calc. for C28H26N2O8Sn: C, 52.78; H, 4.11; N, 4.40; Sn, 16.83. Found: C, 52.70; H, 4.02; N, 4.01; Sn, 18.45. Bis(4-maleimido-benzoato)dihexyltin(IV) (4) White solid, mp 138 °C. Yield: 86%. IR νmax/cm⁻¹: 1596 ν(CO), 1431 ν(CO), Δν: 165, 416 ν(Sn–O), 547 ν(Sn–C), 531 ν(Sn–C), 119mSn Mössbauer (mm s⁻¹): QS: 3.16, IS: 1.33, Γ: 0.94, p: 2.37, 1H NMR (CDCl3) δ 7.8 (d, J 2.0 Hz, 1H, CH), 7.7 (d, J 7.1 Hz, 1H, CH), 7.0 (d, J 7.3 Hz, 1H, CH), 1.4 (t, J 119mSn–H) 99 Hz, 2H, CH₂), 1.8 (m, 2H, CH₂), 1.3 (m, 11H, CH₃), 0.9 (t, J 4.2 Hz, 3H, CH₃); 13C NMR (75 MHz, CDCl₃) δ: 169.6(1C), 132.2(2C), 138.0(3C); 131.0(4C), 136.9(5C), 171.9(6C), 134.1(7C), 27.1(14C), 1J(119mSn–I) 911 Hz), 27.3(15C), 2J(119mSn–I) 235 Hz). Anal. Calc. for C36H30N2O8Sn: C, 54.16; H, 4.55; N, 4.21; Sn, 17.84. Found: C, 54.01; H, 4.45; N, 4.13; Sn, 17.62. Bis(4-maleimido-benzoato)diphenyltin(IV) (5) White solid, mp 83 °C. Yield: 71%. IR νmax/cm⁻¹: 1577 ν(CO), 1425 ν(CO), Δν: 152, 400 ν(Sn–O), 530 ν(Sn–C), 511 ν(Sn–C), 119mSn Mössbauer (mm s⁻¹): QS: 3.38, IS: 1.04, Γ: 0.97, p: 0.96, p: 3.25. 1H NMR (CDCl₃) δ 7.8 (d, J 2.0 Hz, 1H, CH), 7.6 (d, J 7.1 Hz, 1H, CH), 7.1 (d, J 7.3 Hz, 1H, CH), 7.0 (t, J 119mSn–H) 102 Hz, 2H, CH₂), 1.6 (m, 2H, CH₂) 0.8 (t, J 4.2 Hz, 3H, CH₃); 13C NMR (75 MHz, CDCl₃) δ: 168.3(1C), 130.5(2C), 137.4(3C); 132.3(4C), 138.7(5C), 170.0(6C), 139.0(7C), 129.1(8C), 1J(119mSn–I) 326 Hz), 122.1(9C), 1J(119mSn–I) 154 Hz), 135.4(10C), 1J(119mSn–I) 198 Hz), 130.1(11C), 1J(119mSn–I) 123 Hz). Anal. Calc. for C42H32N2O8Sn: C, 57.90; H, 3.14; N, 3.97; Sn, 16.83. Found: C, 57.79; H, 3.09; N, 3.74; Sn, 16.59. Bis(4-maleimido-benzoato)diethyltin(IV) (6) White solid, mp 142 °C. Yield: 89%. IR νmax/cm⁻¹: 1593 ν(CO), 1424 ν(CO), Δν: 169, 416 ν(Sn–O), 564 ν(Sn–C), 534 ν(Sn–C), 119mSn Mössbauer (mm s⁻¹): QS: 3.29, IS: 1.54, Γ: 0.98, p: 0.92, p: 2.13, 1H NMR (CDCl₃) δ 7.7 (d, J 2.0 Hz, 1H, CH), 7.7 (d, J 7.1 Hz, 1H, CH), 7.1 (d, J 7.3 Hz, 1H, CH), 3.0 (s, 2H, CH₂), 7.7 (m, 1H, CH), 7.3 (d, J 5.2 Hz, 1H, CH), 7.4 (m, 1H, CH); 13C NMR (75 MHz, CDCl₃) δ: 168.3(1C), 130.5(2C), 137.4(3C); 134.3(4C), 137.2(5C), 169.8(6C), 136.7(7C), 20.6(8C), 1J(119mSn–I) 401 Hz), 142.1(9C), 1J(119mSn–I) 174 Hz), 127.5(10C), 1J(119mSn–I) 223 Hz), 129.4(11C), 125.3(12C), 1J(119mSn–I) 231 Hz). Anal. Calc. for C30H26N2O8Sn: C, 58.96; H, 3.57; N, 3.82; Sn, 16.19. Found: C, 58.74; H, 3.57; N, 3.76; Sn, 15.97.
4-maleimido-benzoic acid (7)

White solid, mp 109 °C. Yield: 89%. IR ν max/cm⁻¹: 1680 ν(COO)a, 1405 ν(COO)a, 1275 ν(COO)a, 1244 ν(COO)a, 1192 ν(COO)a, 1130 ν(COO)a, 1115 ν(COO)a, 1058 ν(COO)a, 1020 ν(COO)a, 971 ν(COO)a, 956 ν(COO)a, 922 ν(COO)a, 878 ν(COO)a, 837 ν(COO)a, 790 ν(COO)a, 743 ν(COO)a, 700 ν(COO)a, 667 ν(COO)a, 620 ν(COO)a, 594 ν(COO)a, 554 ν(COO)a, 490 ν(COO)a, 440 ν(COO)a, 420 ν(COO)a, 392 ν(COO)a, 373 ν(COO)a, 360 ν(COO)a, 340 ν(COO)a, 320 ν(COO)a, 290 ν(COO)a, 252 ν(COO)a, 230 ν(COO)a, 210 ν(COO)a, 190 ν(COO)a, 170 ν(COO)a, 150 ν(COO)a, 130 ν(COO)a, 110 ν(COO)a, 90 ν(COO)a, 70 ν(COO)a, 50 ν(COO)a, 30 ν(COO)a, 20 ν(COO)a, 10 ν(COO)a, 0 ν(COO)a. 1H NMR (CDCl₃) δ 7.9 (d, J = 2.0 Hz, 1H, CH), 7.6 (d, J = 7.1 Hz, 1H, CH), 7.2 (t, J = 7.3 Hz, 1H, CH). 13C NMR (75 MHz, CDCl₃): δ: 166.8(1C), 131.6(2C), 135.7(3C); 129.6(4C), 140.4(5C), 165.9(6C), 135.1(7C). Anal. Calc. for C₁₁H₇NO₄: C, 60.83; H, 3.25; N, 6.45. Found: C, 59.68; H, 3.15; N, 6.26.

In vitro anti-leishmanial activity

All promastigote cultures of both the reference and local Pakistani leishmanial strains were maintained in blood agar based bi-phasic Evans modified Tobies medium supplemented with RPMI-1640 with 25 mmol L⁻¹ TES at 25 °C. Leishmanial strains in promastigote stage that were used include L. major (JISH118), L. major (MHOM/PK/88/DESTO), L. tropica (K27), L. infantum (LEM3437), L. mex. (LV4) and L. donovani (H43).

Viability test

Parasites in the promastigote stage were transferred from Evans modified to RPMI-1640 supplemented with RPMI-1640 without FBS or bovine serum (FBS) and 1% sterile human urine, buffered 25 °C. Parasites in the promastigote stage were transferred from Evans modified to RPMI-1640 supplemented with RPMI-1640 with 25 mmol L⁻¹ TES at 25 °C. Leishmanial strains in promastigote stage that were used include L. major (JISH118), L. major (MHOM/PK/88/DESTO), L. tropica (K27), L. infantum (LEM3437), L. mex. (LV4) and L. donovani (H43).

Results and Discussion

The ligand 4-maleimido-benzoic acid and its diorganotin(IV) complexes were synthesized by a general procedure as shown in Figure 1. Analytical data for the complexes confirmed the 1:2 metal-ligand stoichiometry. All compounds were quite stable with good yield (70-92%) and were soluble in organic solvents. Elemental analysis data were found to be in good agreement with calculated contents.
Equation (2), in its turn, employing \( ^1J[^{119}\text{Sn}–^{13}\text{C}] \) values, provided C–Sn–C angles of 176º, 179º, 174º and 177º respectively for 1-4, confirming the trans-octahedral arrangement.

\(^{119}\text{Sn} \) NMR spectroscopy plays a significant role in the determination of geometry around tin atoms.\(^{13} \) \(^{119}\text{Sn} \) NMR chemical shifts of 1-4 (-220.3, -208.5, -213.6 and -221.8 ppm) were comparable with earlier reports describing octahedral geometry.\(^{14} \) On the other hand, 5 showed a broad singlet at \(-114.3 \) ppm, indicating the existence of an equilibrium between penta and hexa-coordination states describing a skew trapezoidal geometry (Figure 2b), while 6 displayed a resonance peak at \(-44.6 \) ppm characteristic of a tetrahedral SnIV centre; in this case, coordination may be lost due to the size of the benzyl group.\(^{15} \) The coupling constant \( ^1J[^{119}\text{Sn}–^{13}\text{C}] \) furnished a typical trend, \( ^1J >> ^2J < ^3J \), which confirmed the tetrahedral geometry of 6 (Figure 2c).\(^{15} \) These results were comparable to the solid state geometrical behavior of the complexes, confirming the 1:2 metal-to-ligand stoichiometry in the solid as well as in CDCl\(_3\) for all complexes.

**Bioactivity**

Table 1 contains the *in vitro* anti-leishmanial activity data of 1-7 and two reference drugs used clinically (Amphotericin B and Pentamidine). These displayed an anti-leishmanial activity trend as \( 7 < 1 < 2 < 3 < 4 < 5 < 6 >> A \) and B (A: Amphotericin B, B: Pentamidine). The results obtained have been depicted in Figure 3, which suggests that the nature and size of the R group attached to SnIV affect the *in vitro* anti-leishmanial activity.

For highlighting this statement, the mean values of the average IC\(_{50} \) for compounds 1-7 against each leishmanial strain have been plotted *versus* the percent CH of R groups attached to SnIV in Figure 4. The percent CH for compounds 1-7 were calculated as:

\[
\text{Percent CH} = \frac{[C_n \times (12.011) + H_n \times (1.0079)]}{\text{Molecular Mass of the Complex}} \times 100
\]

where \( n \) is the number of carbon or hydrogen atoms in R groups.

Figure 4 shows that the lethality increases almost linearly (and therefore IC\(_{50} \) decreases) with the increase in percent CH of the R groups. Some deviations in the case of smaller alkyl groups have been observed, which may be attributed to variation in the conformational behavior and distribution of complexes between phases. Literature reveals that organotin(IV) compounds formed with ligands containing carboxylic groups (–COO–Sn bonds) have proved to be more biologically active; the use of 4-maleimido-benzoic acid as ligand increases the hydrolysability of the organotin(IV) precursors due to formation of the Sn–O bonds.\(^{16} \) This property of the ligand permits attack of the hydrolysed \( \{\text{R}_2\text{SnIV}\}_2^+ \) moieties on the target cells, thereby enhancing the anti-leishmanial activity.\(^{16} \)

On the other hand, the function of the R group is to determine the extent of activity; in this work, the trend was observed that the increase in the size of R groups made

**Figure 2.** Geometrical trends in compounds 1-6 (a) trans-octahedral; (b) skew-trapezoidal; (c) tetrahedral; and numbering scheme for \(^1\text{H} \) and \(^{13}\text{C} \) NMR.

**Table 1.** *In vitro* anti-leishmanial effect of 1-7 and standard drugs (A: Amphotericin B and B: Pentamidine, IC\(_{50} \) in μg mL\(^{-1} \))
the \( \{R\text{Sn}^{IV}\}^2+ \) moieties more lipophilic, resulting in the decrease of IC\(_{50}\) when compared with reference drugs (A and B) and starting organotin(IV) reagents. Conclusively, we can say that the bulkiness of the attached R group/percent CH values and polar character of carboxylic group of 4-maleimido-benzoic acid are interlinked with each other, and enhances the polarity C–Sn and O–Sn bonds in 1-6. A study is being carried out for the in vivo interactions/mechanism of action of these complexes.

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Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br, as PDF file.

References


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