J. Braz. Chem. Soc., Vol. 20, No. 2, 341-347, 2009.
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0103 - 5053 $6.00+0.00

Article

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 R_2SnL_2 (onde R: Me(**1**), Et(**2**), Pr(**3**), Bu(**4**), Ph(**5**), Bz(**6**) e L(**7**): ácido *p-N*-maleimidobenzóico) foram sintetizados neste trabalho. Espectros de absorção no infravermelho e de Mössbauer de ^{119m}Sn no estado sólido revelaram o comportamento bidentado de L em relação ao centro diorganoestanho(IV) nos complexos octaédricos distorcidos. Espectros de RMN de ^{1}H, ^{13}C e ^{119}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. major*, *L. tropica*, *L. infantum*, *L. mex. mex.* e *L. 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 R em $\{R_2Sn^{IV}\}^{2+}$ aumentou a lipofilicidade dos complexos organoestanho(IV), acentuando assim a atividade antileishmania.

Six new diorganotin(IV) esters with the general composition R_2SnL_2 (where R: Me(**1**), Et(**2**), Pr(**3**), Bu(**4**), Ph(**5**), Bz(**6**) and L(**7**): *p-N*-maleimido-benzoic acid) have been synthesized. Solid state FTIR and ^{119m}Sn Mössbauer spectra revealed bidentate behavior of L towards the diorganotin(IV) centre in the distorted octahedral products. ^{1}H, ^{13}C and ^{119}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. major*, *L. tropica*, *L. infantum*, *L. mex. mex.* and *L. 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 R groups in the $\{R_2Sn^{IV}\}^{2+}$ 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.[1] 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.[2] Organotin(IV) compounds are well-known for their manifold implications, such as tumouricidal, bactericidal and fungicidal activities, and for their interesting structural features.[3] 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*

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and *in vivo* as anti-leishmanial agents against *L. donovani*, and considered a good prospect as a therapeutic mediator for leishmaniasis.[4] 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,[4] 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.[5]

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.[5] 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 TENSOR27spectrophotometer 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 ^{57}Co-Pd source, while the zero point of the Doppler velocity scale was determined through the absorption spectra of $CaSnO_3$ (^{119}Sn = 0.5 mg cm^{-2}). The resulting 5×10^5-count spectra were refined to obtain the isomer shift (IS), the nuclear quadrupole splitting (QS), ρ (QS/IS) and the width at half-height of the resonant peaks, Γ (mm s^{-1}). ^{1}H and ^{13}C NMR spectra in deuterated chloroform ($CDCl_3$) were recorded on a multinuclear Brüker Biospin AMX 300 MHz FT NMR spectrometer operating at room temperature (300 MHz for ^{1}H and 75 MHz for ^{13}C); the hydrogen and carbon chemical shifts were measured with respect to $SiMe_4$. ^{119}Sn NMR spectra in $CDCl_3$ were recorded at 186.5 MHz on a Brüker AMX 500 spectrophotometer using 5 mm o.d. tubes and are reported relative to external neat $SnMe_4$ (δ ^{119}Sn = 0 ppm). Important parameters were acquisition time (AQ) of 3.6 s, relaxation time (d1) 0.01 s, sweep width (SW) 4545.45 Hz and number of data points (TD) 32768.

Synthesis of 4-maleimido-benzoic acid

Maleic anhydride (10 g, 0.1 mol L^{-1}) was dissolved in acetic acid (150 mL) and a cold solution of 4-aminobenzoic acid (9.1 g, 0.1 mol L^{-1}) in acetic acid (150 mL) was added to it. This mixture was stirred at room temperature for 3 h resulting in a white precipitate, which was washed several times with cold water and recrystallized from water to give maleamic acid of analytical purity. Maleamic acid (5 g, 0.02 mol L^{-1}) was then suspended in dry toluene (350 mL), and triethylamine (7.5 mL, 0.05 mol L^{-1}) was added to this suspension. The mixture was refluxed with vigorous stirring for 1.5 h with the concomitant removal of water using a Dean-Stark separator. The solvent was removed on a rotary evaporator (Büchi) leaving a hygroscopic solid; HCl was added up to pH 2 and the mixture was extracted with ethyl acetate and dried over anhydrous $MgSO_4$. The ethyl acetate fraction was vacuum dried; the solid mass left was recrystallized from hexane. Figure 1 depicts a general chemical reaction scheme.

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 C_6H_6 and finally recrystallized from CH_2Cl_2.

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

*Bis(4-maleimido-benzoato)dimethyltin(IV) (**1**)*

White solid, mp 158 °C. Yield: 81%. IR ν_{max}/cm^{-1}: 1631 $\nu(COO)_a$, 1447 $\nu(COO)_s$, Δν: 184, 411 ν(Sn–O), 522 $\nu(Sn–C)_a$, 517 $\nu(Sn–C)_s$. ^{119m}Sn Mössbauer (mm s^{-1}): QS: 3.31, IS: 1.32, Γ_1: 0.98, Γ_2: 0.87, ρ: 2.50. ^{1}H NMR ($CDCl_3$) δ 7.8 (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), 0.6 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 175.4(1C), 133.6(2C), 136.8(3C); 129.0(4C), 136.4(5C), 170.0(6C), 135.4(7C), –0.7 (8C, $^1J(^{119}Sn–^{13}C)$ 903 Hz). Anal. Calc. for $C_{24}H_{18}N_2O_8Sn$: C, 49.52; H, 3.04; N, 4.82; Sn, 20.43. Found: C, 49.60; H, 3.12; N, 4.66; Sn, 20.29.

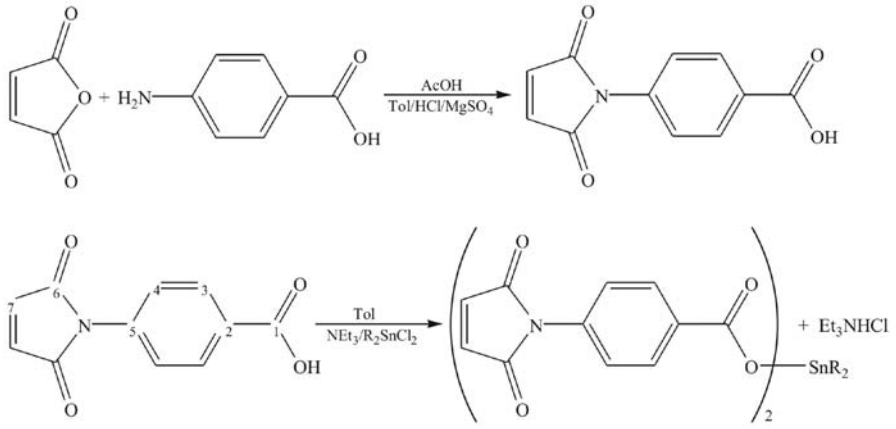

Figure 1. General reaction scheme for the synthesis of 4-maleimido-benzoic acid and its tin^{IV} complexes.

*Bis(4-maleimido-benzoato)diethyltin(IV) (**2**)*

White solid, mp 74 °C. Yield: 83%. IR ν_{max}/cm^{-1}: 1655 $\nu(COO)_a$, 1474 $\nu(COO)_s$, $\Delta\nu$: 181, 409 ν(Sn–O), 537 $\nu(Sn–C)_a$, 523 $\nu(Sn–C)_s$. ^{119m}Sn Mössbauer (mm s^{-1}): QS: 3.44, IS: 1.41, Γ_1: 0.96, Γ_2: 0.84, ρ: 2.43. ^{1}H NMR ($CDCl_3$) δ 7.8 (d, *J* 2.0 Hz, 1H, CH), 7.8 (d, *J* 7.1 Hz, 1H, CH), 7.1 (d, *J* 7.3 Hz, 1H, CH), 0.9 (q, ^2J (^{119}Sn-^{1}H) 100 Hz, 2H, CH_2), 0.8 (t, *J* 4.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.5(1C), 130.1(2C), 137.0(3C); 131.3(4C), 138.2(5C), 172.3(6C), 139.2(7C), 10.7(8C, ^1J(^{119}Sn–^{13}C) 918 Hz), 6.1(9C, ^2J(^{119}Sn–^{13}C) 187 Hz). Anal. Calc. for $C_{26}H_{22}N_2O_8Sn$: C, 51.46; H, 3.64; N, 4.60; Sn, 19.19. Found: C, 51.12; H, 4.37; N, 4.51; Sn, 19.03.

*Bis(4-maleimido-benzoato)dipropyltin(IV) (**3**)*

White solid, mp 94 °C. Yield: 87%. IR ν_{max}/cm^{-1}: 1651 $\nu(COO)_a$, 1483 $\nu(COO)_s$, $\Delta\nu$: 168, 468 ν(Sn–O), 529 $\nu(Sn–C)_a$, 517 $\nu(Sn–C)_s$. ^{119m}Sn Mössbauer (mm s^{-1}): QS: 3.42, IS: 1.28, Γ_1: 1.00, Γ_2: 0.88, ρ: 2.67. ^{1}H NMR ($CDCl_3$) δ 7.8 (d, *J* 2.0 Hz, 1H, CH), 7.9 (d, *J* 7.1 Hz, 1H, CH), 7.0 (d, *J* 7.3 Hz, 1H, CH), 1.1 (t, ^2J (^{119}Sn-^{1}H) 102 Hz, 2H, CH_2), 1.6 (m, 2H, CH_2) 0.8 (t, *J* 4.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.6(1C), 132.2(2C), 138.0(3C); 131.0(4C), 136.9(5C), 171.1(6C), 135.1(7C), 34.2 (8C, ^1J(^{119}Sn–^{13}C) 893 Hz), 20.3(9C, ^2J(^{119}Sn–^{13}C) 196 Hz). Anal. Calc. for $C_{28}H_{26}N_2O_8Sn$: C, 52.78; H, 4.11; N, 4.40; Sn, 18.63. Found: C, 52.70; H, 4.02; N, 4.01; Sn, 18.45.

*Bis(4-maleimido-benzoato)dibutyltin(IV) (**4**)*

White solid, mp 138 °C. Yield: 86%. IR ν_{max}/cm^{-1}: 1596 $\nu(COO)_a$, 1431 $\nu(COO)_s$, $\Delta\nu$: 165, 415 ν(Sn–O), 547 $\nu(Sn–C)_a$, 531 $\nu(Sn–C)_s$. ^{119m}Sn Mössbauer (mm s^{-1}): QS: 3.16, IS: 1.33, Γ_1: 1.01, Γ_2: 0.94, ρ: 2.37. ^{1}H NMR ($CDCl_3$) 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, ^2J (^{119}Sn-^{1}H) 99 Hz, 2H, CH_2), 1.8 (m, 2H, CH_2), 1.3 (m, 11H, CH_2), 0.9 (t, *J* 4.2 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) 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(^{119}Sn–^{13}C) 911 Hz), 27.3(15C, ^2J(^{119}Sn–^{13}C) 235 Hz). Anal. Calc. for $C_{30}H_{30}N_2O_8Sn$: 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 ν_{max}/cm^{-1}: 1577 $\nu(COO)_a$, 1425 $\nu(COO)_s$, $\Delta\nu$: 152, 400 ν(Sn–O), 530 $\nu(Sn–C)_a$, 511 $\nu(Sn–C)_s$. ^{119m}Sn Mössbauer (mm s^{-1}): QS: 3.38, IS: 1.04, Γ_1: 0.97, Γ_2: 0.96, ρ: 3.25. ^{1}H NMR ($CDCl_3$) 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), 7.8 (d, *J* 7.7 Hz, 1H, CH), 7.6 (t, *J* 7.7 Hz, 1H, CH), 1.3 (t, *J* 7.7 Hz, 1H, CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.0(1C), 129.9(2C), 136.8(3C); 132.3(4C), 138.7(5C), 170.0(6C), 139.0(7C), 129.1(8C, ^1J(^{119}Sn–^{13}C) 326 Hz), 122.1(9C, ^2J(^{119}Sn–^{13}C) 154 Hz), 135.4(10C, ^3J(^{119}Sn–^{13}C) 198 Hz), 130.1(11C, ^4J(^{119}Sn–^{13}C) 123 Hz). Anal. Calc. for $C_{34}H_{22}N_2O_8Sn$: 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)dibenzyltin(IV) (**6**)*

White solid, mp 142 °C. Yield: 89%. IR ν_{max}/cm^{-1}: 1593 $\nu(COO)_a$, 1424 $\nu(COO)_s$, $\Delta\nu$: 169, 416 ν(Sn–O), 564 $\nu(Sn–C)_a$, 534 $\nu(Sn–C)_s$. ^{119m}Sn Mössbauer (mm s^{-1}): QS: 3.29, IS: 1.54, Γ_1: 0.98, Γ_2: 0.92, ρ: 2.13. ^{1}H NMR ($CDCl_3$) 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, CH_2), 7.7 (m, 1H, CH), 7.3 (d, *J* 5.2 Hz, 1H, CH), 7.4 (m, 1H, CH); ^{13}C NMR (75 MHz, $CDCl_3$) 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(^{119}Sn–^{13}C) 401 Hz), 142.1(9C, ^2J(^{119}Sn–^{13}C) 174 Hz), 127.5(10C, ^3J(^{119}Sn–^{13}C) 223 Hz), 129.4(11C), 125.3(12C, ^4J(^{119}Sn–^{13}C) 231 Hz). Anal. Calc. for $C_{36}H_{26}N_2O_8Sn$: 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 $\nu(COO)_a$, 1405 $\nu(COO)_s$, $\Delta\nu$: 275, 3375-2870 ν(O–H). ^{1}H NMR ($CDCl_3$) 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), ^{13}C NMR (75 MHz, $CDCl_3$) 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 $C_{11}H_7NO_4$: 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. mex.* (LV4) and *L. donovani* (H43).

Viability test

Parasites in the promastigote stage were transferred from Evans modified to RPMI-1640 supplemented with 5% fetal bovine serum (FBS) and 1% sterile human urine, buffered with 25 mmol L^{-1} TES, pH 7.2 (complete medium). They were grown in bulk at 25 °C and then centrifuged at 2500 rev. *per* min for 10 min; early log phase promastigotes were collected. The parasites were washed twice with RPMI (without FBS or urine) and resuspended in the complete medium to achieve a final concentration of 10^6 parasites *per* mL. In order to get the 50% mortality concentration (IC_{50}), serial dilutions of the test compounds were performed in 96-well microtiter plate. Subsequently, 10^5 promastigotes in 100 µL of culture medium were added to each well and the plate was incubated at 25 ºC for 72 h. Negative controls (culture without test compounds) were on the same plate. At the end of the incubation time, the plate was shaken mechanically over a plane shaker and parasites were counted with the help of a hemocytometer. Dose-dependent viability curves were obtained.

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.

Molecular structure

Solid-state FT IR spectra were recorded in the spectral range of 4000-400 cm^{-1} and important ν_a(COO), ν_s(COO), ν_a(Sn–C), ν_s(Sn–C), ν_a(Sn–O) vibrational frequencies were observed in this region. The complexation of $\{R_2Sn^{IV}\}^{2+}$ moieties with 4-maleimido-benzoic acid was confirmed by the absence of the broad band (**1**-**6**) of ν(OH) due to COOH group (**7**) in the spectral range of 3000-2600 cm^{-1}.[6] The imide ν(N–C=O) band in the range of 1700-1710 cm^{-1} remained unchanged, which ruled out the interaction of Sn^{IV} with imide CO.[6]

It is reported in the literature that the difference ($\Delta\nu$) between ν_a(COO) and ν_s(COO) is important in predicting the coordinating ability of the ligand; in complexes **1**-**5**, $\Delta\nu$ was less than 200 cm^{-1}, which indicated the bidentate nature of 4-maleimido-benzoic acid (Figure 2a).[7] In the spectrum of **5**, a characteristic sharp peak at 450 cm^{-1} confirmed the Sn–Ph bond.[7] In addition, bands of medium intensity observed in the spectral ranges of 570-545 cm^{-1} and 490-430 cm^{-1} confirmed the presence of Sn–C {ν_a(Sn–C), ν_s(Sn–C)} and Sn–O bonds respectively.[4]

^{119m}Sn Mössbauer spectroscopy provides useful information on the geometry around the tin atom in the solid state.[8] In particular, quadropole splitting (QS) values often allow the discrimination between tetra- and hypercoordination of Sn^{IV} centres; each of these being identified by characteristic value ranges (tetrahedral: 2.01-2.5 mm s^{-1}, trigonalbipyramidal: 3.0-4.0 mm s^{-1}, *cis*-octahedral: 1.7-2.2 mm s^{-1}, *trans*-octahedral: 3.0-4.5 mm s^{-1}).[7] For diorganotin(IV) dicarboxylates, the ρ values (QS/IS) play an important role in the prediction of the geometry around the tin centre; it is reported in the literature that if the ρ value is greater than 2.1, the dirganotin(IV) dicarboxylates possess a *trans*-octahedral geometry around the tin atom. Hence, in this work, ρ values strongly suggest a *trans*-octahedral geometry for **1**-**4** (Figure 2a).[9]

The $CDCl_3$ NMR spectra of **1**-**6** exhibited the expected resonances arising from the organotin(IV) moieties and hydrogens of 4-maleimido-benzoic acid.[10] The coupling constants $^1J[^{119}Sn–^{13}C]$ and $^2J[^{119}Sn–^1H]$ yield important structural information; the magnitude of these coupling constants was consistent with a six-coordinate tin centre in an octahedral arrangement, indicating a 1:2 metal-to-ligand stoichiometry.[11] Howard's equations (1 and 2) were successfully applied for the estimation of C–Sn–C angle; equation (1) yielded 182º, 186º and 175º, indicating octahedron for compounds **2**-**4**.[12]

$$\theta(C–Sn–C) = 2.28\ ^2J[^{119}Sn–^1H] – 46.4 \quad (1)$$

$$\theta(C–Sn–C) = 0.178\ ^1J[^{119}Sn–^{13}C] + 14.7 \quad (2)$$

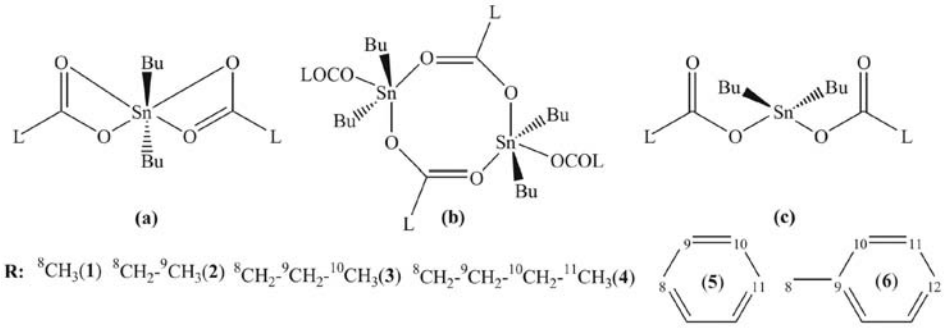

Figure 2. Geometrical trends in compounds **1-6** (a) *trans*-octahedral; (b) skew-trapezoidal; (c) tetrahedral; and numbering scheme for ^{1}H and ^{13}C NMR.

Equation (2), in its turn, employing $^{1}J[^{119}Sn–^{13}C]$ values, provided C–Sn–C angles of 176º, 179º, 174º and 177º respectively for **1-4**, confirming the *trans*-octahedral arrangement.

^{119}Sn NMR spectroscopy plays a significant role in the determination of geometry around tin atoms.[13] ^{119}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 Sn^{IV} centre; in this case, coordination may be lost due to the size of the benzyl group.[15] The coupling constant $[^{1}J(^{119}Sn–^{13}C)]$ furnished a typical trend, *i.e.*, $^{1}J >> {}^{2}J < {}^{3}J$, 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 Sn^{IV} affect the *in vitro* anti-leishmnial 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 Sn^{IV} in Figure 4. The percent CH for compounds **1-7** were calculated as:

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

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 $\{R_2Sn^{IV}\}^{2+}$ 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

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})

Leishm. strain	Compound							**A**	**B**
	(**1**)	(**2**)	(**3**)	(**4**)	(**5**)	(**6**)	(**7**)		
L. major	144	151	127	101	89	66	202	128	203
L major (Pak.)	225	177	142	123	100	95	184	101	147
L. tropica	88	76	44	23	11	03	107	175	203
L. mex. mex.	112	110	87	69	65	49	142	126	167
L. donovani	100	58	54	26	14	08	116	131	108

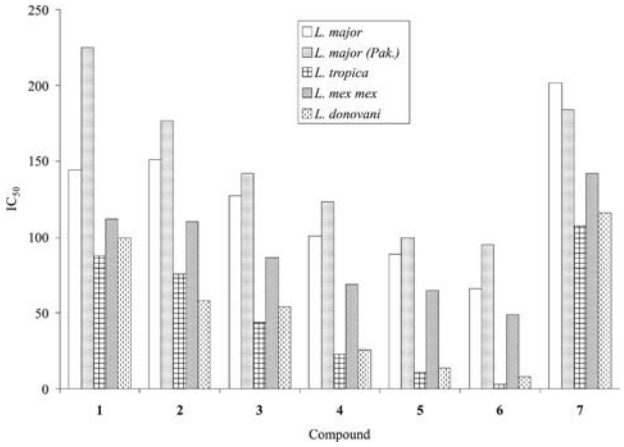

Figure 3. Effect of R groups on anti-leishmanial activity (IC_{50}) of **1-6.**

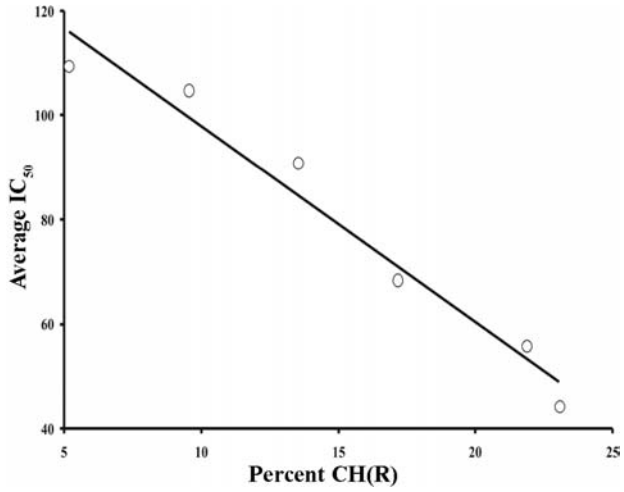

Figure 4. Effect of bulkier R groups (percent CH) on anti-leishmanial activity (IC_{50}).

the $\{R_2Sn^{IV}\}^{2+}$ 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.

Acknowledgment

This work was carried out with the financial support of Gomal University, D. I. Khan, Pakistan, (Research Project No. 717-29/DF/GU).

Supplementary Information

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

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Received: August 6, 2007
Web Release Date: January 22, 2009