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Synthesis, structure and biological activity of diorganotin derivatives with pyridyl functionalized bis(pyrazol-1-yl)methanes

Fang-Lin Li[a], Hai-Bin Song[b], Bin Dai[a] and Liang-Fu Tang[b]*

Three pyridyl functionalized bis(pyrazol-1-yl)methanes, namely 2-[(4-pyridyl)methoxyphenyl] bis(pyrazol-1-yl)methane (L^1), 2-[(4-pyridyl)methoxyphenyl]bis(3,5-dimethylpyrazol-1-yl)methane (L^2) and 2-[(3-pyridyl)methoxyphenyl]bis(pyrazol-1-yl)methane (L^3) have been synthesized by the reactions of (2-hydroxyphenyl)bis(pyrazol-1-yl)methanes with chloromethylpyridine. Treatment of these three ligands with R_2SnCl_2 (R = Et, *n*-Bu or Ph) yields a series of symmetric 2 : 1 adducts of $(L)_2SnR_2Cl_2$ (L = L^1, L^2 or L^3), which have been confirmed by elemental analysis and NMR spectroscopy. The crystal structures of $(L^2)_2Sn(n\text{-}Bu)_2Cl_2\cdot 0.5C_6H_{14}$ and $(L^3)_2SnEt_2Cl_2$ determined by X-ray crystallography show that the functionalized bis(pyrazol-1-yl)methane acts as a monodentate ligand through the pyridyl nitrogen atom, and the pyrazolyl nitrogen atoms do not coordinate to the tin atom. The cytotoxic activity of these complexes for Hela cells *in vitro* was tested.

Supporting information may be found in the online version of this article.

Keywords: bis(pyrazol-1-yl)methane; organotin; pyridyl; biological activity

Introduction

Organotin derivatives have been widely used in industrial and agricultural fields as catalysts or biocides.[1] Among these organotin derivatives, diorganotin derivatives containing a bidentate nitrogen donor ligand [R_2SnX_2(N-N)] have attracted considerable attention due to their potential biological applications. For example, many such complexes have been synthesized and tested for their antitumor activity.[2–10] Moreover, their antitumor activity significantly depends on the Sn–N bond distance. For active complexes, the average Sn–N bond distance is usually longer than 2.39 Å.[6] As a flexible bidentate ligand, bis(pyrazol-1-yl)methane has been found to act as a good donor to the organotin acceptor.[11] The interactions between bis(pyrazol-1-yl)methanes with monodentate and multidentate organotin Lewis acids have also been extensively investigated.[12–18] In recent years, the modification of bis(pyrazol-1-yl)methanes by organic functional groups on the bridging carbon atom has drawn extensive attention owing to the versatile coordination chemistry presented by these new heteroscorpionate ligands,[11,19,20] which encourages us to investigate the interactions of these functionalized ligands with organotin acceptors. In the present work, three pyridyl functionalized bis(pyrazol-1-yl)methanes were synthesized and their reactions with diorganotin dichloride were carried out. These newly synthesized diorganotin derivatives of bis(pyrazol-1-yl)methanes display significant antitumor activity *in vitro* against Hela cells.

Experimental

Materials and Measurements

Solvents were dried by standard methods and distilled prior to use. The reactions involving organotin derivatives were carried out under a water-free atmosphere. Multinuclear NMR spectra were obtained with a Bruker 400 spectrometer using $CDCl_3$ as solvent unless otherwise noted, and the chemical shifts were reported in ppm with respect to reference standards (internal $SiMe_4$ for ^{1}H NMR and ^{13}C NMR spectra, external $SnMe_4$ for ^{119}Sn NMR). Elemental analyses were carried out on an Elementar Vairo EL analyzer. Melting points were measured with an X-4 digital micro melting-point apparatus and are uncorrected. (2-Hydroxyphenyl)bis(pyrazol-1-yl)methane and (2-hydroxyphenyl)bis(3,5-dimethylpyrazol-1-yl)methane[21] were prepared by the published methods.

Synthesis of 2-[(4-Pyridyl)methoxyphenyl]bis(pyrazol-1-yl) methane (L^1)

KOH (4.03 g, 72 mmol) was added to a solution of (2-hydroxyphenyl)bis(pyrazol-1-yl)methane (7.21 g, 30 mmol) in 130 ml of ethanol. The mixture was stirred for 30 min at room temperature, and then 4-chloromethylpyridine hydrochloride (4.92 g, 30 mmol) was added. After stirring for 30 min, the reaction mixture was heated at reflux for 18 h. After cooling to room temperature, water (50 ml) was added. The water solution was extracted with CH_2Cl_2 (3 × 60 ml). The organic layers were combined and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to give red brown oil. This oil was recrystallized from anhydrous ether and treated with activated charcoal to yield white crystals of L^1. Yield:

* *Correspondence to: Liang-Fu Tang, Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, People's Republic of China. E-mail: lftang@nankai.edu.cn*

a *School of Chemistry and Chemical Engineering, Shihezi University, Shihezi 832003, Xinjiang, People's Republic of China*

b *Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, People's Republic of China*

6.33 g (64%); m.p. 127–129 °C. ^{1}H NMR: δ 5.06 (s, 2H, CH_2), 6.33 (t, J = 2.4 Hz, 2H, H^4 of pyrazole), 6.83 (d, J = 7.7 Hz, 1H, C$_6H_4$), 6.90 (d, J = 8.3 Hz, 1H, C$_6H_4$), 7.00 (t, J = 7.6 Hz, 1H, C$_6H_4$), 7.34–7.36 (m, 1H, C$_6H_4$), 7.06, 7.40 (d, J = 5.2 Hz, d, J = 2.0 Hz, 2H, 2H, H^3 and H^5 of pyrazole), 7.66 (d, J = 4.6 Hz, 2H, C$_5H_4$N), 8.66 (d, J = 4.6 Hz, 2H, C$_5H_4$N), 8.08 (s, 1H, C*H*) ppm. ^{13}C NMR: δ 68.1 (CH_2), 73.5 (*C*H), 106.3 (C^4 of pyrazole), 111.8, 121.1, 121.6, 124.7, 128.1, 129.4, 131.0, 140.7, 145.3, 150.0, 155.0 (C$_6$H$_4$, C$_5$H$_4$N as well as C^3 and C^5 of pyrazole) ppm. Anal. calcd for $C_{19}H_{17}N_5$O.0.25Et$_2$O: C, 68.65; H, 5.62; N, 20.02. Found: C, 68.79; H, 6.12; N, 20.40%.

Synthesis of 2-[(4-Pyridyl)methoxyphenyl]bis(3,5-dimethyl pyrazol-1-yl)methane (L^2)

This ligand was obtained similarly using (2-hydroxyphenyl) bis(3,5-dimethylpyrazol-1-yl)methane instead of (2-hydroxyphenyl)bis(pyrazol-1-yl)methane as described above for **L^1**. Yield: 57%; m.p. 150–152 °C. ^{1}H NMR: δ 2.04, 2.18 (s, s, 6H, 6H, CH_3), 4.96 (s, 2H, CH_2), 5.84 (s, 2H, H^4 of pyrazole), 6.69 (d, J = 7.6 Hz, 1H, C$_6H_4$), 6.82 (d, J = 8.2 Hz, 1H, C$_6H_4$), 6.92 (t, J = 7.6 Hz, 1H, C$_6H_4$), 7.26 (t, J = 7.7 Hz, 1H, C$_6H_4$), 7.03 (d, J = 5.2 Hz, 2H, C$_5H_4$N), 8.51 (d, J = 5.2 Hz, 2H, C$_5H_4$N), 7.72 (s, 1H, C*H*) ppm. ^{13}C NMR: δ 11.2, 13.4 (3 or 5-CH_3), 68.0 (CH_2), 70.6 (*C*H), 106.6 (C^4 of pyrazole), 111.5, 121.2, 121.4, 125.4, 128.3, 130.0, 140.3, 145.7, 147.8, 149.8, 155.1 (C$_6$H$_4$, C$_5$H$_4$N as well as C^3 and C^5 of pyrazole) ppm. Anal. calcd for $C_{23}H_{25}N_5$O: C, 71.29; H, 6.50; N, 18.07. Found: C, 70.96; H, 6.63; N, 18.34%.

Synthesis of 2-[(3-Pyridyl)methoxyphenyl]bis(pyrazol-1-yl) methane (L^3)

This ligand was obtained similarly using 3-chloromethylpyridine hydrochloride instead of 4-chloromethylpyridine hydrochloride as described above for **L^1**. Yield: 76%; m.p. 153–154 °C. ^{1}H NMR: δ 5.02 (s, 2H, CH_2), 6.30 (t, J = 2.4 Hz, 2H, H^4 of pyrazole), 6.81 (d, J = 7.2 Hz, 1H, C$_6H_4$), 6.95–6.99 (m, 2H, C$_6H_4$), 7.22–7.25 (m, 1H, C$_6H_4$), 7.35 (dd, J = 1.5 Hz, J = 7.8 Hz, 1H, C$_5H_4$N), 7.38, 7.62 (d, J = 2.3 Hz, d, J = 1.5 Hz, 2H, 2H, H^3 and H^5 of pyrazole), 7.39–7.41 (m, 1H, C$_5H_4$N), 8.42 (d, J = 1.6 Hz, 1H, C$_5H_4$N), 8.54 (dd, J = 1.3 Hz, J = 4.8 Hz, 1H, C$_5H_4$N), 8.00 (s, 1H, C*H*) ppm. ^{13}C NMR: δ 67.5 (CH_2), 73.5 (*C*H), 106.2 (C^4 of pyrazole), 111.8, 121.5, 123.5, 124.8, 128.0, 129.4, 130.9, 131.8, 135.0, 140.7, 148.6, 149.5, 155.2 (C$_6$H$_4$, C$_5$H$_4$N as well as C^3 and C^5 of pyrazole) ppm. Anal. calcd for $C_{19}H_{17}N_5$O.0.25Et$_2$O: C, 68.65; H, 5.62; N, 20.02. Found: C, 68.49; H, 5.20; N, 20.24%.

Synthesis of (L^1)$_2$SnEt$_2$Cl$_2$ (1)

To a solution of **L^1** (0.33 g, 1 mmol) in 40 ml of ether and 10 ml of CH_2Cl_2, the solution of Et_2SnCl_2 (0.25 g, 1 mmol) in 15 ml of ether was added at room temperature. The reaction mixture was continuously stirred for 10 h, during which a precipitate gradually formed. The precipitate was filtered off, washed with ether (3 × 20 ml) and dried *in vacuo* to give white solids of **1**. Yield: 0.42 g (90%); m.p. 149–151 °C. ^{1}H NMR: δ 1.28 (t, J = 7.9 Hz, 3H, CH_3), 1.82 (q, J = 7.9 Hz, 2H, SnCH_2), 5.11 (s, 2H, OCH_2), 6.34 (s, br, 2H, H^4 of pyrazole), 6.83 (d, J = 7.6 Hz, 1H, C$_6H_4$), 6.91 (d, J = 8.3 Hz, 1H, C$_6H_4$), 7.01 (t, J = 7.6 Hz, 1H, C$_6H_4$), 7.36–7.38 (m, 1H, C$_6H_4$), 7.21, 7.41 (d, J = 5.6 Hz, d, J = 2.4 Hz, 2H, 2H, H^3 and H^5 of pyrazole), 7.66, 8.79 (s, br, d, J = 5.3 Hz, 2H, 2H, C$_5H_4$N), 8.09 (s, 1H, C*H*) ppm. ^{13}C NMR: δ 10.3 (CH_3), 27.4 (SnCH_2), 67.8 (OCH_2), 73.5 (*C*H), 106.4 (C^4 of pyrazole), 111.7, 121.9, 124.8, 128.1, 129.3, 129.4, 131.1, 140.8, 147.7, 148.8, 154.8 (C$_6$H$_4$, C$_5$H$_4$N as well as C^3 and C^5 of pyrazole) ppm. ^{119}Sn NMR: δ −91.2, −138.7 ppm. Anal. calcd for $C_{42}H_{44}Cl_2N_{10}O_2$Sn: C, 55.40; H, 4.87; N, 15.38. Found: C, 55.41; H, 4.60; N, 15.00%.

Synthesis of (L^1)$_2$Sn(*n*-Bu)$_2$Cl$_2$ (2)

This complex was obtained similarly using (*n*-Bu)$_2$SnCl$_2$ instead of Et_2SnCl_2 as described above for complex **1**. After stirring for 10 h at room temperature, the solution was concentrated to *ca* 5 ml, and 5 ml of hexane was added to give white solids of **2**. Yield: 93%; m.p. 134–136 °C. ^{1}H NMR: δ 0.84 (t, J = 7.3 Hz, 3H, CH_3), 1.28–1.34 (m, 2H, SnCH_2CH_2CH_2), 1.64–1.67 (m, 2H, SnCH_2CH_2CH_2), 1.81–1.85 (m, 2H, SnCH_2CH_2CH_2), 5.14 (s, 2H, OCH_2), 6.34 (s, br, 2H, H^4 of pyrazole), 6.83 (d, J = 7.6 Hz, 1H, C$_6H_4$), 6.93 (d, J = 8.3 Hz, 1H, C$_6H_4$), 7.01 (t, J = 7.6 Hz, 1H, C$_6H_4$), 7.36–7.38 (m, 1H, C$_6H_4$), 7.23, 7.41 (d, J = 5.2 Hz, s, br, 2H, 2H, H^3 and H^5 of pyrazole), 7.66, 8.75 (s, br, d, J = 5.0 Hz, 2H, 2H, C$_5H_4$N), 8.10 (s, 1H, C*H*) ppm. ^{13}C NMR: δ 13.6 (CH_3), 26.2, 27.6, 34.4 (SnCH_2CH_2CH_2), 67.8 (OCH_2), 73.5 (*C*H), 106.5 (C^4 of pyrazole), 111.7, 121.8, 121.9, 124.7, 128.1, 129.4, 131.1, 140.8, 148.0, 148.4, 154.7 (C$_6$H$_4$, C$_5$H$_4$N as well as C^3 and C^5 of pyrazole) ppm. ^{119}Sn NMR: δ −91.0, −138.1 ppm. Anal. calcd for $C_{46}H_{52}Cl_2N_{10}O_2$Sn.$CH_2Cl_2$: C, 53.68; H, 5.18; N, 13.32. Found: C, 54.04; H, 5.15; N, 13.05%.

Synthesis of (L^1)$_2$SnPh$_2$Cl$_2$ (3)

This complex was obtained similarly using Ph_2SnCl_2 instead of Et_2SnCl_2 as described above for complex **1**. Yield: 95%; m.p. 172–174 °C. ^{1}H NMR: δ 5.07 (s, 2H, CH_2), 6.32 (t, J = 1.5 Hz, 2H, H^4 of pyrazole), 6.82 (d, J = 7.2 Hz, 1H, C$_6H_4$), 6.91 (d, J = 8.4 Hz, 1H, C$_6H_4$), 7.01 (t, J = 7.5 Hz, 1H, C$_6H_4$), 7.34–7.37 (m, 1H, C$_6H_4$), 7.39–7.50 (m, 5H, SnC$_6H_5$), 7.10, 7.64 (d, J = 5.4 Hz, s, br, 2H, 2H, H^3 and H^5 of pyrazole), 7.72–7.75, 8.53 (m, d, J = 6.0 Hz, 2H, 2H, C$_5H_4$N), 8.06 (s, 1H, C*H*) ppm. ^{13}C NMR: δ 67.7 (CH_2), 73.5 (*C*H), 106.5 (C^4 of pyrazole), 111.7, 121.8, 121.9, 124.8, 127.9, 128.2, 128.4, 129.4, 129.6, 131.1, 135.6, 140.8, 148.7, 148.8, 154.7 (C$_6$H$_4$, C$_5$H$_4$N as well as C^3 and C^5 of pyrazole) ppm. ^{119}Sn NMR: δ −400.2 ppm. Anal. calcd for $C_{50}H_{44}Cl_2N_{10}O_2$Sn.0.25$CH_2Cl_2$: C, 58.72; H, 4.36; N, 13.63. Found: C, 58.87; H, 4.81; N, 13.38%.

Synthesis of (L^2)$_2$SnEt$_2$Cl$_2$ (4)

To a solution of **L^2** (0.39 g, 1 mmol) in 40 ml of ether, the solution of Et_2SnCl_2 (0.25 g, 1 mmol) in 15 ml of ether was added at room temperature. The reaction mixture was continuously stirred for 10 h, during which a precipitate gradually formed. The precipitate was filtered off, washed with ether (3 × 20 ml) and dried *in vacuo* to give white solids of **4**. Yield: 0.43 g (84%); m.p. 140–143 °C. ^{1}H NMR: δ 1.28 (t, J = 7.9 Hz, 3H, CH$_2$CH_3), 1.82 (q, J = 7.9 Hz, 2H, SnCH_2), 2.06, 2.20 (s, s, 6H, 6H, CH_3), 4.95 (s, 2H, OCH_2), 5.77 (s, 2H, H^4 of pyrazole), 6.57 (d, J = 7.6 Hz, 1H, C$_6H_4$), 6.75 (d, J = 8.0 Hz, 1H, C$_6H_4$), 6.87 (t, J = 7.2 Hz, 1H, C$_6H_4$), 7.22 (t, J = 7.6 Hz, 1H, C$_6H_4$), 7.06, 8.58 (s, br, s, br, 2H, 2H, C$_5H_4$N), 7.62 (s, 1H, C*H*) ppm. ^{13}C NMR: δ 10.4 (CH$_2$CH_3), 18.7 (SnCH_2), 11.3, 13.9 (3- or 5-CH_3), 67.8 (OCH_2), 70.7 (*C*H), 106.7 (C^4 of pyrazole), 111.4, 121.7, 121.9, 125.4, 128.4, 130.1, 140.4, 147.8, 148.0, 148.9, 154.9 (C$_6$H$_4$, C$_5$H$_4$N as well as C^3 and C^5 of pyrazole) ppm. ^{119}Sn NMR: δ −91.2, −138.8 ppm. Anal. calcd for $C_{50}H_{60}Cl_2N_{10}O_2$Sn: C, 58.72; H, 5.91; N, 13.70. Found: C, 58.40; H, 6.40; N, 13.46%.

Synthesis of $(L^2)_2Sn(n\text{-}Bu)_2Cl_2$ (5)

This complex was obtained similarly using $(n\text{-}Bu)_2SnCl_2$ instead of Et_2SnCl_2 as described above for complex **4**. Yield: 83%; m.p. 138–140 °C. ^{1}H NMR: δ 0.84 (t, J = 7.6 Hz, 3H, CH_3), 1.28–1.34 (m, 2H, $SnCH_2CH_2CH_2$), 1.61–1.66 (m, 2H, $SnCH_2CH_2CH_2$), 1.79–1.83 (m, 2H, $SnCH_2CH_2CH_2$), 2.06, 2.19 (s, s, 6H, 6H, CH_3), 5.04 (s, 2H, OCH_2), 5.86 (s, 2H, H^4 of pyrazole), 6.66 (d, J = 7.6 Hz, 1H, C_6H_4), 6.84 (d, J = 8.4 Hz, 1H, C_6H_4), 6.94 (t, J = 7.2 Hz, 1H, C_6H_4), 7.30 (t, J = 7.6 Hz, 1H, C_6H_4), 7.19 (d, J = 5.6 Hz, 2H, C_5H_4N), 8.74 (d, J = 5.6 Hz, 2H, C_5H_4N), 7.72 (s, 1H, CH) ppm. ^{13}C NMR: δ 11.3, 13.8 (3 or 5-CH_3), 13.6 (CH_2CH_3), 26.2, 27.6, 33.6 ($SnCH_2CH_2CH_2$), 67.7 (OCH_2), 70.6 (CH), 106.7 (C^4 of pyrazole), 111.4, 121.6, 122.0, 125.4, 128.4, 130.1, 140.3, 147.9, 148.0, 148.6, 154.9 (C_6H_4, C_5H_4N as well as C^3 and C^5 of pyrazole) ppm. ^{119}Sn NMR: δ −91.0, −138.1 ppm. Anal. calcd for $C_{54}H_{68}Cl_2N_{10}O_2Sn$: C, 60.12; H, 6.35; N, 12.98. Found: C, 60.24; H, 6.31; N, 12.75%.

Synthesis of $(L^2)_2SnPh_2Cl_2$ (6)

This complex was obtained similarly using $\mathbf{L^2}$ and Ph_2SnCl_2 instead of $\mathbf{L^1}$ and Et_2SnCl_2, respectively, as described above for complex **1**. Yield: 94%; m.p. 155–157 °C. ^{1}H NMR: δ 2.03, 2.12 (s, s, 6H, 6H, CH_3), 5.02 (s, 2H, CH_2), 5.81 (s, 2H, H^4 of pyrazole), 6.65 (d, J = 7.6 Hz, 1H, C_6H_4), 6.83 (d, J = 8.0 Hz, 1H, C_6H_4), 6.94 (t, J = 7.6 Hz, 1H, C_6H_4), 7.27 (t, J = 7.4 Hz, 1H, C_6H_4), 7.14 (d, J = 6.0 Hz, 2H, C_5H_4N), 8.54 (d, J = 6.0 Hz, 2H, C_5H_4N), 7.28–7.34 (m, 3H, C_6H_5), 7.83–7.86 (m, 2H, C_6H_5), 7.68 (s, 1H, CH) ppm. ^{13}C NMR: δ 11.3, 13.8 (3 or 5-CH_3), 67.6 (OCH_2), 70.6 (CH), 106.7 (C^4 of pyrazole), 111.5, 114.7, 121.8, 122.1, 125.4, 128.3, 128.4, 129.3, 130.2, 135.7, 140.3, 148.0, 148.2, 149.9, 154.7 (C_6H_4, C_5H_4N as well as C^3 and C^5 of pyrazole) ppm. ^{119}Sn NMR: δ −68.6, −476.7 ppm. Anal. calcd for $C_{58}H_{60}Cl_2N_{10}O_2Sn.0.5CH_2Cl_2$: C, 60.51; H, 5.29; N, 12.06. Found: C, 60.22; H, 5.01; N, 11.59%.

Synthesis of $(L^3)_2SnEt_2Cl_2$ (7)

This complex was obtained similarly using $\mathbf{L^3}$ instead of $\mathbf{L^1}$ as described above for complex **1**. Yield: 75%; m.p. 142–144 °C. ^{1}H NMR: δ 1.29 (t, J = 7.8 Hz, 3H, CH_2CH_3), 1.81 (q, J = 7.8 Hz, 2H, $SnCH_2$), 5.08 (s, 2H, OCH_2), 6.31 (s, br, 2H, H^4 of pyrazole), 6.83 (d, J = 7.6 Hz, 1H, C_6H_4), 6.98–7.02 (m, 2H, C_6H_4), 7.37–7.41 (m, 4H, H^3 or H^5 of pyrazole, C_6H_4 and C_5H_4N), 7.54 (d, J = 7.8 Hz, 1H, C_5H_4N), 7.63 (s, br, 2H, H^3 or H^5 of pyrazole), 8.04 (s, 1H, CH), 8.71–8.76 (m, 2H, C_5H_4N) ppm. ^{13}C NMR: δ 10.2 (CH_2CH_3), 26.3 ($SnCH_2$), 67.2 (OCH_2), 73.5 (CH), 106.3 (C^4 of pyrazole), 111.9, 121.7, 124.4, 124.8, 128.1, 129.5, 131.0, 132.9, 136.6, 140.7, 147.3, 148.5, 155.0 (C_6H_4, C_5H_4N as well as C^3 and C^5 of pyrazole) ppm. ^{119}Sn NMR: δ −91.0, −138.8 ppm. Anal. calcd for $C_{42}H_{44}Cl_2N_{10}O_2Sn$: C, 55.40; H, 4.87; N, 15.38. Found: C, 55.23; H, 4.51; N, 15.08%.

Synthesis of $(L^3)_2Sn(n\text{-}Bu)_2Cl_2$ (8)

This complex was obtained similarly using $\mathbf{L^3}$ and $(n\text{-}Bu)_2SnCl_2$ instead of $\mathbf{L^1}$ and Et_2SnCl_2, respectively, as described above for complex **1**. After stirring for 10 h at room temperature, the solution was concentrated to *ca* 5 ml, and 5 ml of hexane was added to give white solids of **8**. Yield: 82%; m.p. 104–106 °C. ^{1}H NMR: δ 0.91 (t, J = 7.3 Hz, 3H, CH_3), 1.26–1.41 (m, 2H, $SnCH_2CH_2CH_2$), 1.73–1.81 (m, 4H, $SnCH_2CH_2CH_2$), 5.06 (s, 2H, OCH_2), 6.31 (t, J = 2.0 Hz, 2H, H^4 of pyrazole), 6.82 (d, J = 7.6 Hz, 1H, C_6H_4), 6.97–7.00 (m, 2H, C_6H_4), 7.31–7.39 (m, 4H, H^3 or H^5 of pyrazole, C_6H_4 and C_5H_4N), 7.48 (d, J = 7.8 Hz, 1H, C_5H_4N), 7.63 (d, J = 1.3 Hz, 2H, H^3 or H^5 of pyrazole), 8.01 (s, 1H, CH), 8.55, 8.64 (s, s, 1H, 1H, C_5H_4N) ppm. ^{13}C NMR: δ 13.6 (CH_3), 26.3, 27.1, 29.0 ($SnCH_2CH_2CH_2$), 67.4 (OCH_2), 73.5 (CH), 106.3 (C^4 or pyrazole), 111.8, 121.6, 124.0, 124.8, 128.1, 129.4, 131.0, 132.5, 135.8, 140.7, 147.9, 148.9, 155.1 (C_6H_4, C_5H_4N as well as C^3 and C^5 of pyrazole) ppm. ^{119}Sn NMR: δ −90.9, −138.1 ppm. Anal. calcd for $C_{46}H_{52}Cl_2N_{10}O_2Sn.CH_2Cl_2$: C, 53.68; H, 5.18; N, 13.32. Found: C, 54.18; H, 5.53; N, 13.59%.

Synthesis of $(L^3)_2SnPh_2Cl_2$ (9)

This complex was obtained similarly using $\mathbf{L^3}$ and Ph_2SnCl_2 instead of $\mathbf{L^1}$ and Et_2SnCl_2, respectively, as described above for complex **1**. Yield: 90%; m.p. 181–183 °C. ^{1}H NMR: δ 5.00 (s, 2H, CH_2), 6.30 (t, J = 1.6 Hz, 2H, H^4 of pyrazole), 6.82 (d, J = 7.5 Hz, 1H, C_6H_4), 6.92 (d, J = 8.3 Hz, 1H, C_6H_4), 6.99 (t, J = 7.6 Hz, 1H, C_6H_4), 7.28–7.31 (m, 1H, C_6H_4), 7.35–7.43, 7.72–7.74 (m, m, 6H, 2H, H^3 or H^5 of pyrazole, C_6H_5 and C_5H_4N), 7.48 (d, J = 7.8 Hz, 1H, C_5H_4N), 7.62 (s, br, 2H, H^3 or H^5 of pyrazole), 7.98 (s, 1H, CH), 8.51 (s, 1H, C_5H_4N), 8.57 (d, J = 4.7 Hz, 1H, C_5H_4N) ppm. ^{13}C NMR: δ 67.2 (CH_2), 73.5 (CH), 106.3 (C^4 of pyrazole), 111.8, 121.7, 124.2, 124.8, 128.1, 129.1, 129.5, 130.6, 131.0, 132.7, 135.3, 136.7, 140.7, 140.9, 147.7, 148.9, 155.0 (C_6H_4, C_5H_4N as well as C^3 and C^5 of pyrazole) ppm. ^{119}Sn NMR (DMSO-d_6): δ −386.3 ppm. Anal. calcd for $C_{50}H_{44}Cl_2N_{10}O_2Sn$: C, 59.66; H, 4.41; N, 13.92. Found: C, 59.67; H, 4.49; N, 13.73%.

Crystal Structure Determinations

Colorless crystals of **5** and **7** suitable for X-ray analyses were obtained by slow diffusion of hexane into their CH_2Cl_2 solutions at room temperature. Crystals of **5** contained one-half of a co-crystallized hexane molecule. Intensity data were collected on a Rigaku Saturn CCD detector equipped with graphite-monochromated Mo-K_α radiation (λ = 0.71073 Å) using the ω scan mode at 113(2) K. Semi-empirical absorption corrections were applied and all calculations were performed using the Crystalclear program.[22] The structures were solved by direct methods and difference Fourier map using SHELXS of the SHELXTL package and refined with SHELXL[23] by full-matrix least-squares on F^2. The propyl group (C25–C27) in one of the butyl groups in **5** was disordered and the site occupation factor of these disordered atoms was adjusted (0.61 for C25–C27 atoms and 0.39 for C25′–C27′ atoms, respectively) to give reasonable thermal parameters. The highest residual peak of electron density was away from the disordered C25 atom (0.65 Å) in **5** and the Sn1 atom (1.46 Å) in **7**, respectively. All non-hydrogen atoms were refined anisotropically. A summary of the fundamental crystal data for **5** and **7** is listed in Table 1.

Cytostatic Activity Evaluation

The cytotoxic activity of ligands $\mathbf{L^1}$–$\mathbf{L^3}$, complexes **1**–**9** and their precursors for Hela cells *in vitro* was assayed by the MTT method.[24] Hela cells were seeded into 96-well plates at a concentration of about 4000 cells/well and were incubated in 5% CO_2 atmosphere at 37 °C for 24 h. Then, these cells were treated with different concentrations of each compound. Six-fold wells per toxicant concentration were set. After further incubation for 4 days, 100 µl of MTT (1.0 mg/ml) was added to each well. After subsequent incubation for an additional 4 h, the culture medium was removed, and 150 µl of DMSO was added to dissolve the insoluble formazan precipitates. The plate was shaken on a plate shaker to ensure complete dissolution. The optical density of each

Table 1. Crystal data and refinement parameters for complexes **5** and **7**

Complex	**5**.0.5C_6H_{14}	**7**
Formula	$C_{57}H_{75}Cl_2N_{10}O_2Sn$	$C_{42}H_{44}Cl_2N_{10}O_2Sn$
Formula weight	1121.86	910.46
Crystal size (mm)	0.16 × 0.12 × 0.08	0.20 × 0.18 × 0.12
Crystal system	Triclinic	Triclinic
Space group	$P\bar{1}$	$P\bar{1}$
a (Å)	8.5974(17)	9.4329(19)
b (Å)	12.429(3)	10.357(2)
c (Å)	15.508(3)	11.570(2)
α (deg)	111.25(3)	69.91(3)
β (deg)	90.07(3)	79.90(3)
γ (deg)	96.95(3)	87.17(3)
V (Å)^3	1531.3(6)	1045.0(3)
Z	1	1
D_c (g cm^{-3})	1.217	1.447
F(000)	587	466
μ (mm^{-1})	0.550	0.788
2θ range (deg)	3.5–50.0	3.8–50.0
No. of unique reflections (R_{int})	5375 (0.058)	3671 (0.080)
No. of observed reflections [$I > 2\sigma(I)$]	4035	3007
No. of parameters	392	260
GOF	1.05	1.04
Residuals R, R_w	0.075, 0.197	0.059, 0.142
Largest difference peak and hole (e Å^{-3})	1.09, −0.93	1.60, −1.42
CCDC number	764853	764854

Table 2. The IC_{50} values of compounds for HeLa cells (10^{-6} mol/l)

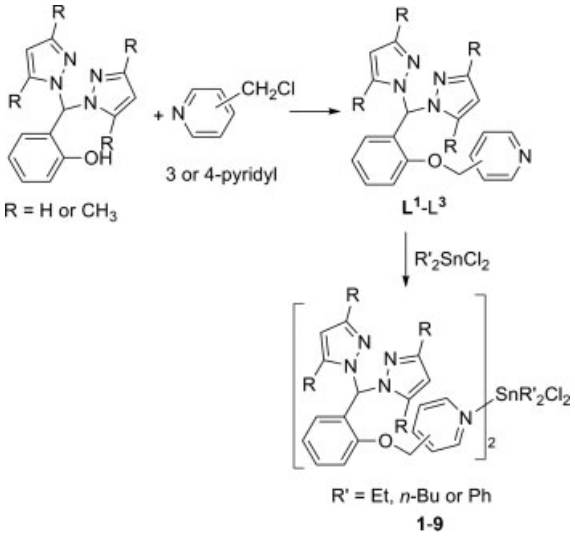
Compound	IC_{50}	Compound	IC_{50}	Compound	IC_{50}
1	2.9	**2**	1.8	**3**	13.1
4	4.9	**5**	4.0	**6**	2.4
7	17.9	**8**	7.2	**9**	13.8
$\mathbf{L^1}$	>100	$\mathbf{L^2}$	>100	$\mathbf{L^3}$	>100
Et_2SnCl_2	18.1	$(n\text{-}Bu)_2SnCl_2$	2.5	Ph_2SnCl_2	4.8
Etoposide	12.6				

well was measured at a wavelength of 490 nm. The cytotoxicity was determined by expressing the mean optical density for drug-treated cells at each concentration as a percentage of that of untreated cells. The activities of compounds were evaluated in terms of their IC_{50} values obtained by linear regression analysis, which are summarized in Table 2.

Results and Discussion

The Modification of Bis(Pyrazol-1-yl)Methanes and Their Reactions

The pyridyl functionalized bis(pyrazol-1-yl)methanes ($\mathbf{L^1}$–$\mathbf{L^3}$) can be easily obtained by the reactions of (2-hydroxyphenyl)bis(pyrazol-1-yl)methanes with chloromethylpyridine under basic conditions (Scheme 1). Treatment of these three ligands with R_2SnCl_2 (R = Et, *n*-Bu or Ph) in a 1 : 1 ratio yields 2 : 1 adducts $(L)_2SnR_2Cl_2$ (**1–9**, L = $\mathbf{L^1}$, $\mathbf{L^2}$ or $\mathbf{L^3}$), which have been characterized by NMR spectroscopy and elemental analyses. The ^{1}H NMR spectroscopic data support the suggested structures. Their ^{1}H NMR spectra exhibit the expected integration values and peak multiplicities for the formulae of 2 : 1 adducts. The chemical shifts of protons and carbons for the ligand moieties in complexes **1–9** are very close to those of the free ligands, suggesting that these complexes are significantly dissociated even in non-coordinating solvent. The ^{119}Sn NMR spectra of these complexes also show the partial loss of the hexacoordinated structures of the 2 : 1 adducts in solution and concomitant formation of new organotin species, such as pentacoordinated 1 : 1 adducts. Two ^{119}Sn NMR signals, corresponding to the values of penta- and hexacoordinated organotin derivatives,[25,26] are observed in the ethyltin complexes **1, 4** and **7**, the butyltin complexes **2, 5** and **8** as well as the phenyltin complex **6**. These dissociative behaviors have been extensively observed in other diorganotin derivatives with nitrogen donor ligands.[2–4,12,15]



Scheme 1. Functionalized bis(pyrazol-1-yl)methanes and their reactions with R_2SnCl_2.

Crystal Structures of Complexes 5 and 7

To verify the coordination mode of these pyridyl functionalized bis(pyrazol-1-yl)methanes, the molecular structures of complexes **5** and **7** were determined by X-ray crystallography, presented in Figs 1 and 2, respectively. The fundamental frameworks in these two complexes are similar to each other. $\mathbf{L^2}$ in complex **5** as well as $\mathbf{L^3}$ in complex **7** acts as a monodentate ligand toward to the tin atom only through the pyridyl nitrogen atom, possibly owing to the stronger donating ability of the pyridyl ring than the pyrazolyl ring.[27] The tin atoms in these two complexes lie on a centre of inversion, and adopt a six-coordinate slightly distorted octahedral geometry with two pyridyl nitrogen atoms, two chlorine atoms and two alkyl carbon atoms in an *all*-trans configuration, similar to those in diorganotin dichloride derivatives with monodentate pyridyl nitrogen donor ligands, such as $Et_2SnCl_2(C_5H_5N)_2$,[28] $Ph_2SnCl_2(C_5H_5N)_2$ [29] and $Ph_2SnCl_2(L')_2$ (L′ = 3,5-dimethyl-4-(4′-pyridyl)pyrazole).[12] The N–Sn–N, Cl–Sn–Cl and C–Sn–C angles

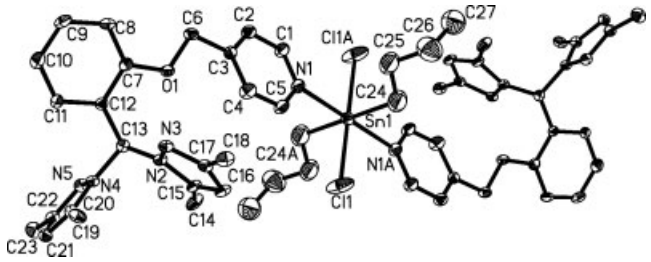


Figure 1. The molecular structure of **5** with the thermal ellipsoids at the 30% probability level. Hydrogen atoms and uncoordinated solvent are omitted for clarity. Selected bond distances (Å) and angles (deg): Sn1–N1, 2.387(3); Sn1–Cl1, 2.547(2); Sn1–C24, 2.101(5); N2–C13, 1.451(5); N4–C13, 1.450(4) Å; and C24–Sn1–N1A, 86.4(2); N1A–Sn1–Cl1, 89.22(9); C24–Sn1–N1, 93.6(2); N1–Sn1–Cl1, 90.78(9); N2–C13–N4, 111.8(3); C6–O1–C7, 118.2(3)°. Symmetry code: $A = 1 - x, -y, -z$.

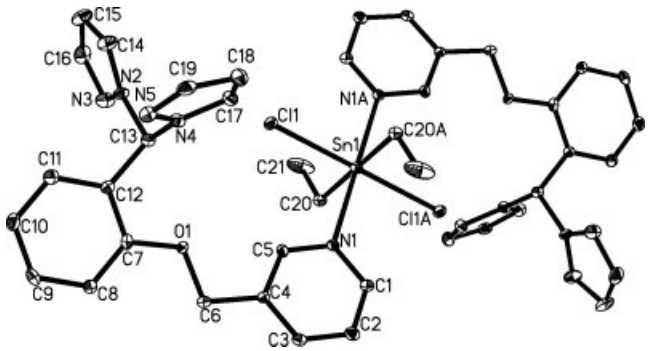


Figure 2. The molecular structure of **7** with the thermal ellipsoids at the 30% probability level. Hydrogen atoms are omitted for clarity. Selected bond distances (Å) and angles (deg): Sn1–N1, 2.399(4); Sn1–Cl1, 2.576(2); Sn1–C20, 2.145(5); N2–C13, 1.467(6); N4–C13, 1.444(6) Å; and C20–Sn1–N1A, 92.1(2); N1–Sn1–Cl1, 90.0(1); N1–Sn1–Cl1A, 89.0(1); N1–Sn1–C20, 87.9(2); N2–C13–N4, 108.6(4); C6–O1–C7, 117.8(3)°. symmetry code: $A = 1 - x, 1 - y, -z$.

are each 180° owing to symmetry in these two complexes. The Sn–N bond distance is 2.387(3) Å in complex **5** and 2.399(4) Å in complex **7**, respectively, longer than those reported in $Ph_2SnCl_2(C_5H_5N)_2$ [2.331(4) and 2.314(4) Å][29] and $Ph_2SnCl_2(L')_2$ (2.365(3) Å),[12] but shorter than those in $Et_2SnCl_2(C_5H_5N)_2$ [2.410(3) and 2.411(3) Å][28] and the corresponding Sn–N(pyridyl) bond distances in diorganotin derivatives with chelating bidentate nitrogen donor ligands, such as in Me_2SnCl_2(PMP) [2.471(4) Å, PMP = 2-(pyrazol-1-ylmethyl)pyridine].[2] The Sn–C [2.101(5) Å in complex **5** and 2.145 (5) Å in complex **7**] and Sn–Cl [2.547(2) Å in complex **5** and 2.576 (2) Å in complex **7**] bond distances are also comparable to those in diorganotin dihalide derivatives with monodentate nitrogen donor ligands.[15,28]

Some weak intermolecular C–H···Cl hydrogen bonding interactions have been observed in the crystal packing of these two complexes, such as C21–H21···Cl1 [H···Cl/C···Cl distances: 2.95(6)/3.80(8) Å; symmetry operation: $1-x, 1-y, 1-z$] in complex **5** and C15–H15···Cl1 [H···Cl/C···Cl distances: 2.83(6)/3.72(8) Å; symmetry operation: $-x, 2-y, -z$] in complex **7**, respectively. The C–H···Cl contacts in each of **5** and **7** result in the formation of linear supramolecular chains in each case.

In Vitro Cytostatic Activity

The data of IC_{50} shown in Table 2 indicate that ligands $\mathbf{L^1}$–$\mathbf{L^3}$ have scarcely any activity against Hela cells, but their complexation with diorganotin chloride leads to a better activity in some complexes. For example, the ethyl derivatives, especially complexes **1** and **4**, exhibit relatively higher activity than their precursors. At the same time, the butyl and phenyl derivatives **2** and **6** have a better activity than their parent diorganotin chloride. Moreover, these four complexes are even more active than etoposide. However, the complexation of the 3-pyridyl ligand with diorganotin chloride results in complexes with a lower activity compared with their parent organotin compounds. The butyltin derivatives (complexes **2, 5** and **8**) are more active than the ethyltin derivatives (complexes **1, 4** and **7**). This behavior has been observed in other diorganotin dihalide adducts containing *N*,*N*′-bidentate ligands.[3,4] The IC_{50} values of complexes **4** and **5** are larger than the corresponding values of complexes **1** and **2**, respectively, reflecting that the methyl groups on the pyrazolyl rings decrease the cytotoxic activities of complexes **4** and **5**. Similar results have been observed in other organotin derivatives with functionalized bis(pyrazol-1-yl)methane.[30] However, the influence of substitutions on the pyrazolyl rings on the activities of diphenyltin derivatives is indistinctive. For example, complex **6** is the most active among these three phenyl derivatives, according to its IC_{50} value. The cytostatic activity discussed herein may be the result of the cooperative effect of 2 : 1 adducts, 1 : 1 adducts and the ligands, owing to the partial dissociation of complexes **1**–**9** in solution shown by their NMR spectra.

In conclusion, three pyridyl functionalized bis(pyrazol-1-yl)methanes (L) were synthesized by the reactions of (2-hydroxyphenyl)bis(pyrazol-1-yl)methanes with chloromethylpyridine. Treatment of these three ligands with R_2SnCl_2 (R = Et, *n*-Bu or Ph) yielded 2 : 1 adducts $(L)_2SnR_2Cl_2$, in which the pyridyl functionalized bis(pyrazol-1-yl)methane acted as a monodentate ligand through the pyridyl nitrogen atom, and the pyrazolyl nitrogen atoms did not coordinate to the tin atom. Some complexes exhibited good cytotoxicities for Hela cells *in vitro*.

Supporting information

Supporting information may be found in the online version of this article.

Acknowledgments

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