Heterobimetallic complexes containing Sn(IV) and Pd(II) with 4-(2-Hydroxyethyl)piperazine-1-carboxylic acid: Synthesis, characterization and biological activities

Shabbir Hussain, Saqib Ali, Saira Shahzadi and Muhammad Shahid

Cogent Chemistry (2015), 1: 1029038
Heterobimetallic complexes containing Sn(IV) and Pd(II) with 4-(2-Hydroxyethyl)piperazine-1-carbodithioic acid: Synthesis, characterization and biological activities

Shabbir Hussain¹, Saqib Ali²*, Saira Shahzadi²* and Muhammad Shahid³

Abstract: Four heterobimetallic derivatives of the type, [R₂(Cl)SnL]PdCl₂ (R = n-Bu: 1)/[R₃SnL]PdCl₂ (R = Me: 2; n-Bu: 3; Ph: 4) where L = 4-(2-hydroxyethyl)piperazine-1-carbodithioic acid, have been synthesized by refluxing 4-(2-hydroxyethyl)piperazine-1-carbodithioic acid with R₂SnCl₂/R₃SnCl in 1:1 M/L ratio and then stirring with PdCl₂ at room temperature. The complexes have been characterized by various spectroscopic (IR, ¹H and ¹³C NMR, and EI-MS) techniques and thermogravimetric analysis. FTIR data demonstrated the bidentate binding of dithiocarbamate group. The NMR data demonstrated 4-coordinated geometry in solution. The complex 4 exhibits the interaction with salmon sperm DNA (SS-DNA) with significant hypochromic effect and suggests intercalating mode of binding. The complex 2 was found most active for inhibition of alkaline phosphates enzyme (ALPs). The complexes 1–4 showed significant antimicrobial activities as compared to the inactive free ligand precursor. The in vitro hemolytic activity showed that average lysis of human red blood cells was significantly lower as compared to Triton-X 100 (positive control, 100% lysis).

Keywords: 4-(2-Hydroxyethyl)piperazine-1-carbodithioic acid; Sn(IV); Pd(II); spectroscopy; thermal; DNA interaction; biological activities

© 2015 The Author(s). This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.
1. Introduction

Considerable attention has been focused on the synthesis of heterobimetallic complexes owing to their potential use in photochemical molecular devices and as light sensitive probes in biological systems (Balzani, Juris, Venturi, Campagna, & Serroni, 1996). It has been observed that heterobimetallic complexes containing both electron-deficient and electron-rich transition metal ions behave in a cooperative manner leading to an enhanced reactivity (Wark & Stephan, 1990). These systems are of great interest because of their structural diversities, molecular magnetism, and electrical conductivities (Robertson & Cronin, 2002). The presence of two metal ions in close proximity mimic the active site of several metalloenzymes and offers an opportunity to study the metal–proteins and metal–enzyme interactions (Magnus, Ton-That, & Carpenter, 1994). Chemistry of organotins attracts interest due to several applications in the form of potential antineoplastic and antituberculosis agents (Zhang, Song, Li, Liu, & Tang, 2007), PVC stabilizers (Arkil & Balköse, 2005), and antitumor drugs (Tabassum & Pettinari, 2006) as well as polymer catalysts (Angiolini et al., 2006). Numerous studies on organotin(IV) complexes have been carried out to explore their biological properties against bacterial, fungal strains, and cancer cells line (Hadjikakou & Hadjiliadis, 2009; Hadjikakou, Ozturk, Xanthopoulou, & Zachariadis, 2008). Recently, significant attention has been focused on the DNA binding properties of dithiocarbamate metal complexes among which Pd(II) and Pt(II) complexes of dithiocarbamates have gained special interest due to their potential antitumor properties (Islami-Moghaddam, Mansouri-Torshizi, Divsalar, & Saboury, 2009; Mansouri-Torshizi, Moghaddam, Divsalar, & Saboury, 2009). Currently, cis-platin is one of the most effective anticancer drug, however, there is an increase of interest in palladium(II) complexes containing N and S donor ligands in recent years, with the aim to synthesize antitumor drugs having a reduced toxicity with respect to cis-platin and analogs (Mansouri-Torshizi et al., 2011).

Keeping in view, wide scope and the applications of organotin(IV)/Pd(II) complexes, we extend our investigations from organotin chemistry (Hussain et al., 2011; Hussain et al., 2012; Shahzadi & Ali, 2008) to the synthesis and characterization of heterobimetallic (Sn, Pd) complexes with 4-(2-hydroxyethyl)piperazine-1-carbodithioic acid. The complexes have been characterized by various spectroscopic techniques and thermogravimetric analyses. DNA binding and alkaline phosphatase inhibition studies of the complexes were also carried out. The complexes were screened against various strains of bacteria/fungi and their minimal inhibitory concentrations were also evaluated. In vitro hemolytic activities were performed to check their possible toxic effects on human red blood cells.

2. Materials and methods

Dibutyltin dichloride, trimethyltin chloride, tributyltin chloride, triphenyltin chloride, palladium chloride, and carbon disulfide were purchased from Sigma-Aldrich (USA) and used without any further purification. 2-(1-Piperazinyl)ethanol was purchased from Merck (Germany). AR grade solvents of Merck (methanol) and Lab-scan (DMSO) origin were used. Methanol was dried before use by a standard procedure (Aramagre & Chai, 2003). p-Nitrophenyl phosphate hexahydrate (pNPP), diethanolamine, and magnesium chloride were purchased from Sigma-Aldrich and used as such. Human serum was used as a source of alkaline phosphatase. Nutrient agar and potato dextrose agar were procured from Oxoid, Hampshire, UK.

The samples were taken in capillary tube and their melting points were recorded by using an electrochemical melting point apparatus Stuart SMP3 and are uncorrected. Infrared spectra were recorded by a Perkin-Elmer-100 FTIR spectrophotometer in the range of 4,000–250 cm⁻¹. The percentage composition of C, H, N, and S were determined by using CHNS-932 elemental analyzer, Leco (USA). The ¹H and ¹³C NMR spectral measurements were made at 300 and 75 MHz, respectively, by Bruker ARC 300 MHz-FT-NMR spectrometer. The EI mass spectrum was recorded using Thermo Fisher Exactive Orbitrap instrument. TGA spectra were obtained by TGA-7 Perkin-Elmer USA under nitrogen atmosphere.

The complexes were tested for their interaction with salmon sperm DNA (SS-DNA) (Sastri, Eswaramoorthy, Giribabu, & Maiya, 2003; Zhang et al., 2011) and alkaline phosphatase enzyme (ALPs) (Malik et al., 2011). The ligand and complexes were screened to check their in vitro response
against various strains of bacteria (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pasturella multocida*) and fungi (*Alternaria alternata*, *Trichoderma harzianum*, and *Aspergillus niger*) by disc diffusion method (Clinical Laboratory Standards Institute (CLSI), 2007) as well as minimum inhibitory concentration (MIC) (Sarker, Nahar, & Kumarasamy, 2007). The activities were performed in an incubator (Sanyo, Germany) and sterilized in an autoclave (Omron, Japan). The minimum inhibitory concentrations were determined in a Micro Quant apparatus (BioTek, USA). Streptomycin and fluconazole were used as standard drugs for antibacterial and antifungal screening tests, respectively. The *in vitro* hemolytic bioassay (Sharma & Sharma, 2001) of the complexes was performed with human red blood cells.

2.1. Synthesis

2.1.1. Synthesis of 4-(2-Hydroxyethyl)piperazine-1-carbodithioic acid (HL)

2-(1-Piperazinyl)ethanol (1 mmol) and CS\(_2\) (1 mmol) were stirred together in methanol (30 mL) in a round bottom two necked flask (250 mL) for 0.5 h. The precipitates obtained were filtered off and dried in air.

2.1.2. General procedure for synthesis of complexes 1–4

4-(2-Hydroxyethyl)piperazine-1-carbodithioic acid (1 mmol) was dissolved in chloroform (150 mL) in a round bottom two necked flask (250 mL) with continuous stirring. Then \(R_2SnCl_2\)/\(R_3SnCl\) (1 mmol) was added as a solid in portions and the solution was refluxed for 3 h. The reaction mixture was cooled at room temperature and then \(PdCl_2\) (1 mmol) solution in distilled water (25 mL) was added drop wise and reaction mixture was further stirred for 2 h with constant stirring resulting in the appearance of orange colored precipitates. The precipitates obtained were filtered off and dried in air. The complexes were recrystallized in acetone and pet. ether (2:1).

2.2. DNA interaction study

A solution of 0.2 g of salmon sperm DNA (SS-DNA) in double deionized water (100 mL) was prepared and kept at 4°C. DNA solution in 20 mM Tris-HCl (pH 7.4) gave the ratio of UV absorbance at 260 and 280 nm, \(A_{260}/A_{280}\) of 1.86 which demonstrated that DNA was free from protein (Zhang et al., 2011). The DNA concentration was found to be \(7.45 \times 10^{-5}\) M via absorption spectroscopy at 260 nm using the molar absorption coefficient of 6,600 M\(^{-1}\) cm\(^{-1}\) (Sastri et al., 2003). From this stock solution, working solutions (10, 19, 27, 35, 42, 48, 54, 59, 64, and 69 μM) were prepared. Solution of the complex with a concentration of \(2 \times 10^{-6}\) M was prepared in 90% DMSO. The UV absorption titrations were performed by keeping the concentration of complexes constant (2 mM) and varying the concentration of DNA. Equivalent solutions of DNA were added to the complex and the reference solutions to eliminate the absorbance of DNA itself. Compound-DNA solutions were incubated at ambient temperature for 30 min before performing the measurements. Absorption spectra were recorded by using the cuvettes of 1 cm path length.

2.3. Alkaline phosphatase activity

The assay of alkaline phosphatase activity was carried out by reported method (Malik et al., 2011) with slight modification. A working substrate was prepared by mixing the four parts of reagent A
(diethanolamine pH = 9.8, 2 mol/dm³ and magnesium chloride 0.5 mmol/dm³) and one part of reagent B (p-nitrophenyl phosphate 50 mmol/dm³). The substrate was incubated for five minutes at 25°C. In a cell cuvette, 2 mL of the substrate was taken and 40 μL of human serum having the activity of 165 IU/L was added. After incubation of 1 min, the absorbance was recorded to check the activity of the enzyme. ALP hydrolyzed the p-NPP and yellow colored p-nitrophenol was produced that absorbed at 405 nm. The assay of the alkaline phosphatase activity gets the advantage of the fact that the enzyme utilizes the non-biological substrate p-nitrophenyl phosphate and it is non-specific.

Then various amounts of complexes were added periodically from the 25 mM stock solution and again incubated for 3 min. Absorbance was recorded again 1, 2, 3, 4, and 5 min thereafter. At the end, the average was taken and the percentage inhibition was calculated.

2.4. Biological activities
Antibacterial and antifungal activities were determined by disc diffusion method (CLSI, 2007). Small filter paper discs (size, 9 mm), each soaked with 100 μL sample solution, were placed flat on a growth medium (nutrient agar for bacteria and potato dextrose agar for fungi) containing microbes and the Petri plates were incubated for 24 h at 37°C for bacterial growth and for 48 h at 28°C for fungal growth. The biologically active samples inhibited the bacterial/fungal growth to form the clear zones, which were measured by a zone reader. The ligand and the products were also evaluated for their MICs by a modified resazurin microtitre plate assay (Sarker et al., 2007). MIC values against fungal strains were noted using nutrient broth medium and resazurin indicator. For fungal strains sabouraud dextrose agar (SDA) medium was used and no indicator was involved.

2.5. Hemolytic activity
Three milliliter of heparinized human blood was freshly obtained from volunteers after consent. Then it was mixed gently, transferred into a 15 mL sterile polystyrene screw-cap tube and centrifuged for 5 min at 850 g. The resultant supernatant mass was poured off and the viscous pellet was washed three times with 5 mL of chilled (4°C) and sterile isotonic phosphate-buffered saline (PBS) of pH 7.4. The washed cells were suspended in 20 mL sterile chilled PBS and counted on a hemocytometer. The blood cell suspension retained on wet ice was diluted for each assay with sterile PBS to 7.068 × 108 cells ml⁻¹. Aliquots of 20 μL of each compound solution were placed aseptically into 2 mL microfuge tubes. For each assay, 0.1% Triton-X 100 was used as a positive control (100% lysis) and PBS as a negative control (0% lysis). Then aliquot of 180 μL diluted blood cell suspension was aseptically placed into each 2 mL tube and gently mixed with a wide mouth pipette tip three times. The tubes were incubated with agitation (80 rpm) at 37°C for 35 min and then placed for 5 min on ice followed by centrifugation at 1,310 g for 5 min. Aliquots of 100 μL of supernatant were collected, placed into a sterile 1.5 mL microfuge tube and then diluted with 900 μL chilled and sterile PBS. All the tubes were maintained on wet ice after dilution. Absorbance was then noted on a microquant at 576 nm. The experiment was performed triplicate. Percent hemolysis was found by following formula (Sharma & Sharma, 2001):

\[
\% \text{ hemolysis} = \frac{\text{Abs (sample absorbance)}}{\text{Abs (control absorbance)}} \times 100
\]

3. Results and discussion
The complexes are stable in air having sharp melting points and are soluble in common organic solvents. The physical data are given in Table 1.

3.1. IR spectroscopy
Infrared spectra of the ligand HL and the complexes 1-4 were recorded in the range of 4,000–250 cm⁻¹. The most important bands are given in Table 2.

A peculiar feature of the IR spectra is the appearance of a broad band at 3,231 and 2,573 cm⁻¹ for υOH and υSH vibrations, respectively, in the free ligand HL. Disappearance of these bands confirm complexation due to deprotonation of –OH and –CSSH moieties for coordination with Sn(IV) and Pd(II), respectively. The υC=S band observed at 1,076 cm⁻¹ in HL was shifted to lower frequency lie in
the range of 980–985 cm$^{-1}$ in the complexes 1–4, indicating the metal sulfur coordination, which is also supported by appearance of $\nu$Pd-S bands in the range of 355–370 cm$^{-1}$ (Singh, Fahmi, & Biyala, 2005). The observed $\nu$(C–N) vibrations (1,429–1,593 cm$^{-1}$) lie between the range as reported earlier (Fregona et al., 2003) for C–N single bonds (1,250–1,360 cm$^{-1}$) and for C=N double bonds (1,640–1,690 cm$^{-1}$). Thus, $\nu$(N-CSS) mode has been shifted to higher frequencies in complexes relative to the HL indicating the appearance of a partial double bond character in CSS$^-$ anion, thus enabling its bidentate coordination with Pd(II) metal (Fregona et al., 2003). New bands were appeared in the range 471–502 cm$^{-1}$ in the complexes 1–4 due to $\nu$Sn–O vibrational mode and the bands in the range 522–542 cm$^{-1}$ and at 254 cm$^{-1}$ were assigned to $\nu$Sn–C vibration in complexes 1–3 and triphenyltin(IV) complex 4, respectively.

### Table 1. Physical data of the complexes 1–4

| Complex No. | Molecular formula | Molecular weight (g/mol) | Yield (%) | Melting point (°C) | Elemental analysis calculated (Found) |
|-------------|-------------------|--------------------------|-----------|-------------------|--------------------------------------|
| HL          | C$_7$H$_{14}$N$_2$OS$_2$ | 206.32                   | 95        | 125–126           | %C 40.75 (40.71) %H 6.84 (6.89) %N 13.58 (13.54) %S 31.08 (31.12) |
| 1           | C$_{15}$H$_{30}$N$_2$OSn$_2$PdCl$_3$ | 649.98                   | 86        | 184–185           | %C 27.72 (27.76) %H 4.65 (4.69) %N 4.31 (4.27) %S 9.86 (9.90) |
| 2           | C$_{10}$H$_{21}$N$_2$OSn$_2$PdCl$_2$ | 545.41                   | 71        | 141–142           | %C 22.02 (22.06) %H 3.88 (3.92) %N 5.14 (5.18) %S 11.76 (11.72) |
| 3           | C$_{19}$H$_{39}$N$_2$OSn$_2$PdCl$_2$ | 671.65                   | 76        | 165–166           | %C 33.98 (33.94) %H 5.85 (5.81) %N 4.17 (4.13) %S 9.55 (9.51) |
| 4           | C$_{25}$H$_{27}$N$_2$OSn$_2$PdCl$_2$ | 731.62                   | 81        | 202–203           | %C 41.04 (41.00) %H 3.72 (3.76) %N 3.83 (3.87) %S 8.76 (8.72) |

### Table 2. IR data (cm$^{-1}$) of complexes 1–4

| Complex No. | $\nu$OH | $\nu$SH | $\nu$C=N | $\nu$C–S | $\nu$Sn–C | $\nu$Sn–Cl | $\nu$Sn–O | $\nu$Pd–S | $\nu$Pd–Cl |
|-------------|---------|---------|----------|----------|-----------|------------|-----------|-----------|-----------|
| HL          | 3231s   | 2573b   | 1409s    | 1076s    | –         | –          | –         | –         | –         |
| 1           | –       | –       | 1505s    | 985s     | 522b      | 336s       | 496s      | 365 m     | 282 m     |
| 2           | –       | –       | 1444s    | 985s     | 542b      | –          | 471w      | 356w      | 279s      |
| 3           | –       | –       | 1429s    | 984s     | 538b      | –          | 483b      | 370 m     | 285 m     |
| 4           | –       | –       | 1593s    | 980s     | 254s      | –          | 502b      | 355w      | 288w      |

Notes: Abbreviations: s = strong; m = medium; w = weak; b = broad.

3.2. $^1$H NMR spectroscopy

The $^1$H NMR data is given in Table 3. The signals were assigned by their distinct multiplicity patterns, resonance intensities, coupling constants, and tin satellites. The number of protons found by integration of peaks in the spectra agreed very well with those calculated from the expected composition.

The singlet resonance occurring at 4.95 ppm and 4.49 ppm was assigned to the –SH and –OH protons, respectively, in the dithiocarbamate ligand (HL). The absence of these resonances (–OH/–SH) in the spectra of the complexes verified bimetallic coordination through deprotonated hydroxyl/dithiocarbamate moieties as also indicated by IR spectroscopy. The methyl protons in complex 2 appeared as singlet at 1.05 ppm with $^3$J(119Sn–1H) coupling constant of 55 Hz. The C–Sn–C bond angle (109°) calculated from $^3$J(119Sn–1H) value (Lockhart, Manders, & Holt, 1986) supports the tetrahedral geometry around tin(IV) in the complex 2. Despite the complex pattern of di- and tri-n-butyl fragments in the spectra, a clear triplet due to terminal methyl group was appeared at 0.85 ppm and 0.87 ppm for the complexes 1 and 3, respectively, with $^3$J(H, H) = 7.2 Hz in each case. Ortho protons absorbed downfield as compared to meta and para protons in the triphenyltin(IV) complex 4 (Hussain et al., 2012).
3.3. \(^{13}\)C NMR spectroscopy

The assignments of –CSS group in investigated compounds is straightforward which is observed in the range of 201.5–202.6 ppm indicating the coordination of sulfur to the tin atom. Table 4 lists the chemical shifts of \(^{13}\)C and tin-carbon coupling constants for complexes 1–4. The \(^{13}\)C NMR chemical shifts due to the phenyl groups are observed at positions comparable to other similar compounds (Al-Hayaly et al., 2005; Bonati & Ugo, 1967). Coordination of the tin atom in complexes 1–4 has been related to \(nJ(119\text{Sn}-13\text{C})\) coupling constants. The \(nJ(119\text{Sn}-13\text{C})\) coupling for complexes 2 and 3 is 522 and 478 Hz, respectively, which is indicative of 4-coordinated geometry (Bonati & Ugo, 1967) in solution state.

3.4. Mass spectrometry

The electron ionization mass spectrum (EI-MS) was recorded for complex 1. The mass spectral data are given in Table 5. The mass spectrum of complex 1 showed no molecular ion (M+) peak.

**Table 3. \(^{1}\)H NMR data of complexes 1–4**

| Proton No. | Chemical shift (ppm) |
|----------------|----------------------|
| HL | 1 | 2 | 3 | 4 |
| 1 | 4.95s | – | – | – | – |
| 2,2' | 3.10–3.13 m | 3.15–3.22 m | 3.13–3.26 m | 3.21–3.35 m | 3.20–3.37 m |
| 3,3' | 2.43–2.65 m | 2.48–2.59 m | 2.50–2.54 m | 2.51–2.56 m | 2.53–2.57 m |
| 4 | 2.91s | 2.93s | 2.92s | 2.91s | 2.92s |
| 5 | 3.50s | 3.52s | 3.51s | 3.53s | 3.51s |
| 6 | 4.49s | – | – | – | – |

**Table 4. \(^{13}\)C NMR data of complexes 1–4**

| Carbon No. | Chemical shift (ppm) |
|----------------|----------------------|
| HL | 1 | 2 | 3 | 4 |
| 1 | 197.4 | 201.8 | 202.6 | 201.5 | 201.7 |
| 2,2' | 58.5 | 58.7 | 58.6 | 58.6 | 58.5 |
| 3,3' | 52.6 | 52.3 | 52.4 | 52.6 | 52.3 |
| 4 | 59.5 | 59.6 | 59.5 | 59.4 | 59.6 |
| 5 | 67.6 | 67.8 | 67.7 | 67.6 | 67.8 |

Notes: \(nJ(119\text{Sn},1\text{H})\) and \(nJ(1\text{H},1\text{H})\) coupling constants are listed in square brackets and parenthesis, respectively; Multiplicity is given as: s = singlet, t = triplet, m = multiplet.
complex 1 loses an ethoxide ion to yield \([\text{C}_13\text{H}_{25}\text{N}_2\text{PdS}_2\text{SnCl}_3]^+\) fragment with \(m/z = 604\) (2%). Primary fragmentation in complex 1 occurs by eliminating \(\text{PdCl}_2\) in the first step and two butene molecules in the next two steps to generate \([\text{C}_7\text{H}_{14}\text{ClN}_2\text{O}_2\text{S}_2\text{Sn}]^+\) fragment at \(m/z = 361\) (4%) which then releases \(\text{H}_2 + \text{HCl}, \text{CS}_2, \text{Sn}, \text{CO}, \text{and } \text{CH}_3\text{CHNH}\) in different steps and ultimately gave the fragment \([\text{C}_3\text{H}_6\text{N}]^+\) at \(m/z = 56\) (66%). Primary decompostion may also generate fragment \([\text{C}_5\text{H}_9\text{ClN}_2\text{PdS}_2]^+\) at \(m/z = 302\) (5%), which further split up into \([\text{PdCl}]^+\) fragment at \(m/z = 143\) (15%) and \([\text{CS}_2]^+\) fragment at \(m/z = 76\) (100).

### 3.5. Thermogravimetric analysis

Thermogravimetric analysis (TGA) of the ligand \(\text{HL}\) and complexes 1, 2, and 4 were performed under \(\text{N}_2\) atmosphere to evaluate their degradation pattern and thermal stability. The theoretically calculated weight loss was compared with experimental results (Table 6). The thermally decomposed data agreed well with the expected chemical composition of the ligand and the complexes. The thermal stability of the complexes was found greater than that of the respective ligand, since the increase in metal content makes a complex more resistant to decomposition (Kalia, Kaushal, Lumba, & Priyanka, 2008). The thermal decomposition resulted in the evolution of coordinated organotin(IV) moieties as reported earlier (Sharma, Kaistha, & Bhatt, 2003; Valla, Bakola-Christianopoulou, Akrivos, Kojic, & Bogdanovic, 2006).

### 3.6. DNA interaction study

For complexes 1–4, DNA binding parameters were evaluated by using absorption spectroscopy. It was found that only the complex 4 exhibits binding with DNA. There exists a single band in the absorption spectrum at 281.5 nm for complex 4. The UV spectrum of complex 4 (Figure 1) showed significant hypochromic effect and suggests intercalating mode of binding. After 24 h, the spectrum was again taken and obtained the same results which confirmed the stability of drug–DNA complex.

### Table 5. Mass spectral data of complex 1

| Complex No. | MS, m/z (%) | 
|-------------|-------------|
| 1 | \([\text{C}_13\text{H}_{25}\text{N}_2\text{PdS}_2\text{SnCl}_3]^+\) 649 (n. o)^, \([\text{C}_13\text{H}_{19}\text{N}_2\text{PdS}_2\text{SnCl}_3]^+\) 604 (2), \([\text{C}_15\text{H}_{30}\text{ClN}_2\text{O}_2\text{S}_2\text{Sn}]^+\) 473 (19), \([\text{C}_15\text{H}_{30}\text{ClN}_2\text{O}_2\text{Sn}]^+\) 417 (13), \([\text{C}_15\text{H}_{30}\text{ClN}_2\text{O}_2\text{Sn}]^+\) 361 (4), \([\text{C}_15\text{H}_{30}\text{ClN}_2\text{O}_2\text{Sn}]^+\) 323 (3), \([\text{C}_15\text{H}_{30}\text{ClN}_2\text{O}_2\text{Sn}]^+\) 302 (5), \([\text{C}_15\text{H}_{30}\text{ClN}_2\text{O}_2\text{Sn}]^+\) 267 (22), \([\text{C}_15\text{H}_{30}\text{ClN}_2\text{O}_2\text{Sn}]^+\) 247 (8), \([\text{C}_15\text{H}_{30}\text{ClN}_2\text{O}_2\text{Sn}]^+\) 209 (10), \([\text{SnCl}]^+\) 155 (12), \([\text{PdCl}]^+\) 143 (15), \([\text{C}_7\text{H}_{14}\text{N}_2\text{O}]^+\) 127 (22), \([\text{C}_7\text{H}_{14}\text{N}_2\text{O}]^+\) 99 (85), \([\text{CS}_2]^+\) 76 (100), \([\text{C}_3\text{H}_6\text{N}]^+\) 56 (66). |

^Molecular ion peak (M+); n. o = not observed.

### Table 6. Thermal decomposition data of complexes 1, 2, and 4

| Complex No. | Molecular formula | Evolved components | % loss calculated/ found | Temperature range (°C) |
|-------------|-------------------|--------------------|--------------------------|------------------------|
| HL | \(\text{C}_7\text{H}_{14}\text{O}_2\text{S}_2\) | \(\text{H}_2\text{O}, 3\text{C}_2\text{H}_4, \text{N}_2, \text{CS}_2\) | 100/98.62 | 73.16–300.6 |
| 1 | \(\text{C}_13\text{H}_{25}\text{N}_2\text{O}_2\text{SnS}_2\text{PdCl}_3\) | \(3\text{C}_2\text{H}_4, 2\text{Bu}, \text{CS}, \text{ClO}, \text{N}_2\) | 49.46/48.49 | 49.77–255.49 |
|  |  | \(\text{SnCl}_2\) | 29.18/29.99 | 255.54–829.88 |
| 2 | \(\text{C}_{10}\text{H}_{21}\text{N}_2\text{O}_2\text{SnS}_2\text{PdCl}_2\) | \(2\text{C}_2\text{H}_4, \text{CC}, \text{N}_2, \text{OH} / 1/2\text{SnCl}_4, 3/2\text{CH}_3\) | 53.67/53.54 | 51.61–338.39 |
|  |  | \(1/2\text{Me}_3\text{Sn}\) | 15.02/15.21 | 379.20–800 |
| 4 | \(\text{C}_{25}\text{H}_{27}\text{N}_2\text{O}_2\text{SnS}_2\text{PdCl}_2\) | \(3\text{C}_6\text{H}_5, 2\text{C}_2\text{H}_4, \text{C}_2\text{H}_4\text{O}, \text{CS}_2, 1/2\text{SnCl}_4, \text{N}_2\) | 77.36/78.40 | 106.11–850 |
|  |  | \(\text{Residue left behind = } 1/2\text{Sn}, \text{Pd}\) |  | |

Residue left behind = none.

Residue left behind = \(\text{PdS}\).

Residue left behind = \(1/2\text{Sn}, \text{Pd}\).
The intrinsic binding constant $K$ for the investigated DNA active complex was calculated in order to compare binding strengths of complex–DNA and ligand–DNA by using Benesi–Hildebrand equation (Ahmad et al., 2007):

\[
\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_D} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_D} \times \frac{1}{K[\text{DNA}]}
\]

where $K$ = binding constant; $A_0$ = absorbance of the drug; $A$ = absorbance of the drug and its complex with DNA; $\varepsilon_G$ = absorption coefficient of the drug; and $\varepsilon_{H-G}$ = absorption coefficient of the drug–DNA complex.

The association constant was obtained from the intercept-to-slope ratios of $A_0/(A - A_0)$ vs. $1/[\text{DNA}]$ plots.

The binding constant was found to be $5.9 \times 10^2$ M$^{-1}$ for complex 4. The Gibb’s free energy of complex 4 was determined by using the following equation:

\[
\Delta G = -RT \ln K
\]

where $R$ is general gas constant ($8.314$ J K$^{-1}$ mol$^{-1}$) and $T$ is the temperature (298 K). The Gibb’s free energy was found to be $-15.8$ for complex 4. The negative value suggests that the interaction of the compound with DNA is a spontaneous process.

### 3.7. Alkaline phosphatase activities (ALPs)

The ligand HL and complexes 1–4 were screened for their ALPs activity (Malik et al., 2011). Ligand was found totally inactive against the enzymatic activity, while complexes 1–4 rendered activity for inhibition of ALPs (Liu, Chou, & Humphreys, 1979). Inhibition of ALPs is because of blockage of active sites of the enzyme by the palladium ion. Actually, the Pd may replace the Zn or Mg metal of the enzyme and hence the enzyme fails to bind with the substrate. It may also be possible that enzyme binds with the palladium complex or palladium ion more efficiently rather than substrate. The exact mechanism is still unknown. Among the complexes 1–4, only complex 2 was the most active one due to smaller size of its organotin(IV) moiety. The complex 4 was found to be least active due to larger size of organotin moiety (Figure 2).

### 3.8. Antimicrobial activities

The ligand and the complexes were screened to check their in vitro response against various strains of bacteria (*E. coli*, *B. subtilis*, *S. aureus*, and *P. multocida*) and fungi (*A. alternata*, *G. lucidum*, *P. notatum*, *T. harzianum* and *A. niger*) by disc diffusion method (CLSI, 2007) and measurement of MIC (Sarker et al., 2007). The data are given in Table 7.
The biological activity was found to depend mainly upon the absence/presence of organotin(IV) fragments in the complexes. The free ligand was found inactive against all the tested organisms as reported earlier (Hussain et al., 2015), whereas the complexes possessed comparable activity as reported in literature (Hussain et al., 2015; Rafiq et al., 2014) for other heterobimetallic complexes. The coordination of the ligand with chlorodi- or trialkyltin(IV) moieties have appreciably enhanced the activities of complexes (Hussain et al., 2014).

3.9. Hemolytic activity

Hemolytic activity of the ligand and complexes 1–4 was performed because, even if a compound exhibits potent antimicrobial activities, its use is not recommended in medicine in the presence of these hemolytic effects. Thus, in vitro hemolytic bioassay of the complexes was performed with human red blood cells and the average lysis was reported with respect to the triton X-100 as positive control (100% lysis) and PBS as negative control (0% lysis). The results are given in Table 8. The complexes

| Complex No. | Zone of inhibition (mm) | MIC value (µg/well) |
|-------------|------------------------|---------------------|
|             | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 |
| E. coli     | 29±0.26 | 20±0.12 | 30±0.31 | 28±0.21 | 30±0.17 | 78 | 39 | 78 | 156 | 1 |
| B. subtilis | 30±0.21 | 21±0.23 | 30±0.28 | 20±0.13 | 31±0.28 | 19 | 19 | 78 | 39 | 2 |
| S. aureus   | 26±0.15 | 16±0.14 | 27±0.19 | 24±0.26 | 31±0.31 | 19 | 19 | 78 | 39 | 78 |
| P. multocida| 30±0.26 | 19±0.15 | 21±0.21 | 26±0.29 | 29±0.28 | 9  | 78 | 78 | 19 | 39 |
| A. alternata| 18±0.15 | 20±0.12 | 20±0.09 | 18±0.12 | 38±0.29 | 104| 104| 52 | 26 | 26 |
| G. lucidum  | 25±0.23 | 28±0.22 | 27±0.13 | 23±0.16 | 41±0.21 | 52 | 13 | 0.4| 26 |
| P. notatum  | 20±0.19 | 33±0.21 | 28±0.26 | 20±0.14 | 45±0.31 | 26 | 13 | 0.8| 3  | >0.4 |
| T. harzianum| 23±0.09 | 26±0.18 | 19±0.21 | 22±0.13 | -     | 13 | -  | 13 | 6  | 416 |

Notes: Data are expressed as the mean ± standard deviation of samples analyzed individually in triplicate. 0 = No activity, 5–10 = Activity present, 11–25 = Moderate activity, 26–40 = Strong activity. Different letters in superscripts in a same row indicate significant differences in activities. a = maximum activity, b = intermediate activity, c = minimum activity, ab = activity between maximum and intermediate, and bc = activity between intermediate and minimum; Streptomycin and Fluconazole are standard antibacterial and antifungal drugs, respectively.
1–4 exhibit lower hemolytic activity as compared to their free ligand HL, however, the activity was found significantly lower as compared to triton-X 100. The lowest (11.98%) and highest activity (57.41%) was found for the complex 4 and complex 2, respectively.

4. Conclusion
We have synthesized heterobimetallic complexes with 4-(2-hydroxyethyl)piperazine-1-carboxylic acid. The ligand and complexes have been characterized by IR, NMR, mass spectrometry, and TGA.

IR data show bidentate nature of dithiocarbamate group in complexes 1–4. NMR data show 4-coordinated geometry in solution. The complex 4 exhibits the interaction with salmon sperm DNA (SS-DNA) with hypochromic effect and suggests intercalating mode of binding. In alkaline phosphatase enzyme assay, only the complex 2 was found active. The complexes exhibited significant antimicrobial activities as compared to free ligand. The hemolytic activity shows that the complexes exhibit comparatively higher hemolytic activity as compared to the free ligand.

Funding
Shabbir Hussain thanks the HEC, Islamabad, Pakistan, for financial support under the PhD Fellowship Scheme Batch-IV (PIN Code: 074-3160-P4/4-362).

Author details
Shabbir Hussain1
E-mail: shabchem@yahoo.com
Saqib Ali2
E-mail: dirsa54@yahoo.com
Saira Shazidi3
E-mail: sairashahzadi@hotmail.com
Muhammad Shahid3
E-mail: mshahidus@yahoo.com
1 Department of Chemistry, GC University, Faisalabad, Pakistan.
2 Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan.
3 Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan.

Citation information
Cite this article as: Heterobimetallic complexes containing Sn(IV) and Pd(II) with 4-(2-Hydroxyethyl)piperazine-1-carboxylic acid: Synthesis, characterization and biological activities, Shabbir Hussain, Saqib Ali, Saira Shazidi & Muhammad Shahid, Cogent Chemistry (2015), 1: 1029038.

Cover image
Source: Authors.

References
Ahmad, M. S., Hussain, M., Hanif, M., Ali, S., & Mirza, B. (2007). Synthesis, chemical characterization and biological screening for cytotoxicity and antitumor activity of Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid. Molecules, 12, 2348–2363. http://dx.doi.org/10.3390/molecules12102348
Al-Hayaly, L. J., Buttrus, N. H., Tarq, F., & Al-Allaf, T. A. K. (2005). Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid: X-ray structure of Chlorodimethyl-(4-hydroxymethyl)vinyltin(IV). Journal of Applied Science (Jordan), 17, 64–70.
Angiolini, L., Caretti, D., Mazzucchetti, L., Salatelli, E., Willems, R., & Biesemans, M. (2006). Cross-linked polystyrene resins containing triorganotin 4-vinylbenzoates: Assessment of their catalytic activity in transesterification reactions. Journal of Organometallic Chemistry, 691, 3043–3052. http://dx.doi.org/10.1016/j.jorganchem.2006.03.016
Arks, E., & Balköse, D. (2005). Thermal stabilisation of poly(vinyl chloride) by organotin compounds. Polymer Degradation and Stability, 88, 46–51. http://dx.doi.org/10.1016/j.polymdegradstab.2004.02.021

Cover image
Source: Authors.

References
Ahmad, M. S., Hussain, M., Hanif, M., Ali, S., & Mirza, B. (2007). Synthesis, chemical characterization and biological screening for cytotoxicity and antitumor activity of Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid. Molecules, 12, 2348–2363. http://dx.doi.org/10.3390/molecules12102348
Al-Hayaly, L. J., Buttrus, N. H., Tarq, F., & Al-Allaf, T. A. K. (2005). Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid: X-ray structure of Chlorodimethyl-(4-hydroxymethyl)vinyltin(IV). Journal of Applied Science (Jordan), 17, 64–70.
Angiolini, L., Caretti, D., Mazzucchetti, L., Salatelli, E., Willems, R., & Biesemans, M. (2006). Cross-linked polystyrene resins containing triorganotin 4-vinylbenzoates: Assessment of their catalytic activity in transesterification reactions. Journal of Organometallic Chemistry, 691, 3043–3052. http://dx.doi.org/10.1016/j.jorganchem.2006.03.016
Arks, E., & Balköse, D. (2005). Thermal stabilisation of poly(vinyl chloride) by organotin compounds. Polymer Degradation and Stability, 88, 46–51. http://dx.doi.org/10.1016/j.polymdegradstab.2004.02.021

Cover image
Source: Authors.

References
Ahmad, M. S., Hussain, M., Hanif, M., Ali, S., & Mirza, B. (2007). Synthesis, chemical characterization and biological screening for cytotoxicity and antitumor activity of Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid. Molecules, 12, 2348–2363. http://dx.doi.org/10.3390/molecules12102348
Al-Hayaly, L. J., Buttrus, N. H., Tarq, F., & Al-Allaf, T. A. K. (2005). Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid: X-ray structure of Chlorodimethyl-(4-hydroxymethyl)vinyltin(IV). Journal of Applied Science (Jordan), 17, 64–70.
Angiolini, L., Caretti, D., Mazzucchetti, L., Salatelli, E., Willems, R., & Biesemans, M. (2006). Cross-linked polystyrene resins containing triorganotin 4-vinylbenzoates: Assessment of their catalytic activity in transesterification reactions. Journal of Organometallic Chemistry, 691, 3043–3052. http://dx.doi.org/10.1016/j.jorganchem.2006.03.016
Arks, E., & Balköse, D. (2005). Thermal stabilisation of poly(vinyl chloride) by organotin compounds. Polymer Degradation and Stability, 88, 46–51. http://dx.doi.org/10.1016/j.polymdegradstab.2004.02.021

Cover image
Source: Authors.

References
Ahmad, M. S., Hussain, M., Hanif, M., Ali, S., & Mirza, B. (2007). Synthesis, chemical characterization and biological screening for cytotoxicity and antitumor activity of Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid. Molecules, 12, 2348–2363. http://dx.doi.org/10.3390/molecules12102348
Al-Hayaly, L. J., Buttrus, N. H., Tarq, F., & Al-Allaf, T. A. K. (2005). Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid: X-ray structure of Chlorodimethyl-(4-hydroxymethyl)vinyltin(IV). Journal of Applied Science (Jordan), 17, 64–70.
Angiolini, L., Caretti, D., Mazzucchetti, L., Salatelli, E., Willems, R., & Biesemans, M. (2006). Cross-linked polystyrene resins containing triorganotin 4-vinylbenzoates: Assessment of their catalytic activity in transesterification reactions. Journal of Organometallic Chemistry, 691, 3043–3052. http://dx.doi.org/10.1016/j.jorganchem.2006.03.016
Arks, E., & Balköse, D. (2005). Thermal stabilisation of poly(vinyl chloride) by organotin compounds. Polymer Degradation and Stability, 88, 46–51. http://dx.doi.org/10.1016/j.polymdegradstab.2004.02.021

Cover image
Source: Authors.

References
Ahmad, M. S., Hussain, M., Hanif, M., Ali, S., & Mirza, B. (2007). Synthesis, chemical characterization and biological screening for cytotoxicity and antitumor activity of Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid. Molecules, 12, 2348–2363. http://dx.doi.org/10.3390/molecules12102348
Al-Hayaly, L. J., Buttrus, N. H., Tarq, F., & Al-Allaf, T. A. K. (2005). Organotin (IV) derivatives of 3,4-methylenedioxy 6-nitrophenylpropionic acid: X-ray structure of Chlorodimethyl-(4-hydroxymethyl)vinyltin(IV). Journal of Applied Science (Jordan), 17, 64–70.
Angiolini, L., Caretti, D., Mazzucchetti, L., Salatelli, E., Willems, R., & Biesemans, M. (2006). Cross-linked polystyrene resins containing triorganotin 4-vinylbenzoates: Assessment of their catalytic activity in transesterification reactions. Journal of Organometallic Chemistry, 691, 3043–3052. http://dx.doi.org/10.1016/j.jorganchem.2006.03.016
Arks, E., & Balköse, D. (2005). Thermal stabilisation of poly(vinyl chloride) by organotin compounds. Polymer Degradation and Stability, 88, 46–51. http://dx.doi.org/10.1016/j.polymdegradstab.2004.02.021

Cover image
Source: Authors.
Hussain, S., Bukhari, I. H., Ali, S., Shahzadi, S., Shahid, M., & Munawar, K. S. (2015). Synthesis and spectroscopic and thermogravimetric characterization of heterobimetallic complexes with Sn(IV) and Pd(II); DNA binding, alkaline phosphatase inhibition and biological activity studies. Journal of Coordination Chemistry, 68, 662–677. http://dx.doi.org/10.1080/09598972.2014.994515

Islam-Moghaddam, M., Mansouri-Torshizi, H., Divsalar, A., & Saboury, A. A. (2009). Synthesis, characterization, cytotoxic and DNA binding studies of dinilmine Platinum(II) and Palladium(II) complexes of short hydrocarbon chain ethyldithiocarbamate ligand. Journal of the Iranian Chemical Society, 6, 552–569. http://dx.doi.org/10.1007/BF03246535

Kalia, S. B., Kaushal, G., Lumba, K., & Priyanka. (2008). Thermodynamical investigations of 4-methylpiperazine-1-carboxylic acid ligand and its ion(III), cobalt(II), copper(II) and zinc(II) complexes. Journal of Thermal Analysis and Calorimetry, 91, 609–613. http://dx.doi.org/10.1007/s10973-007-8331-1

Liu, T. Z., Chou, L. Y., & Humphreys, M. H. (1979). Inhibition of intestinal alkaline phosphatase by palladium. Toxicology Letters, 4, 433–438. http://dx.doi.org/10.1016/0378-4274(79)90108-5

Magnus, K. A., Ton-That, H., & Carpenter, J. E. (1994). Recent developments in conducting or magnetic crystalline assemblies. Coordination Chemistry Reviews, 129, 138–145.

Mansouri-Torshizi, H., Moghadam, M. I., Divsalar, A., & Saboury, A. A. (2009). Dinilmine Platinum(II) and Palladium(II) complexes of dithiocarbamate derivative as potential antitumor agents: Synthesis, characterization, cytotoxicity, and detail DNA-binding studies. Journal of Biomolecular Structure and Dynamics, 26, 575–586. http://dx.doi.org/10.1080/07391102.2009.10507273

Mansouri-Torshizi, H., Saedifar, M., Ghasemi, Z. Y., Khojast, M., Divsalar, A., & Saboury, A. A. (2011).MES-bis(N,N′-dithiocarboxylato-κS2) structural studies of zinc(II) complexes with ethyldithiocarbamate ligand. Journal of the Iranian Chemical Society, 8, 662–677. http://dx.doi.org/10.1080/155331701200281026

Pellerito, C., Nagy, L., Pellerito, L., & Szorcisk, A. (2006). Biological activity studies on organotin(IV) complexes and parent compounds. Journal of Organometallic Chemistry, 691, 1733–1747. http://dx.doi.org/10.1016/j.jorganchem.2005.12.025

Rafiq, M., Ali, S., Shahzadi, S., Shahid, M., Sharma, S. K., & Qanungo, K. (2014). Synthesis, characterization, and biological activities of homo- and heterobimetallic complexes of Sn(IV) and Pd(II) with 2-mercapto-5-methyl benzimidazole. Journal of the Iranian Chemical Society, 11, 169–178. http://dx.doi.org/10.1007/s13738-013-0287-4

Robertson, N., & Cronin, L. (2002). Metal bis-1,2-dithiolene complexes in conducting or magnetic crystalline assemblies. Coordination Chemistry Reviews, 227, 93–127. http://dx.doi.org/10.1016/S0010-8545(01)00457-X

Sarker, S. D., Nahar, L., & Kumarasamy, Y. (2007). Microtubule plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods, 42, 321–324. http://dx.doi.org/10.1016/j.jchem.2007.01.006

Sastri, C. V., Eswaramoorthy, D., Giribabu, L., & Maitoy, B. G. (2003). DNA interactions of new mixed-ligand complexes of cobalt(III) and nickel(II) that incorporate modified phenanthroline ligands. Journal of Inorganic Biochemistry, 94, 138–145. http://dx.doi.org/10.1016/S0022-1139(03)00462-5

Shahzadi, S., & Ali, S. (2008). Structural chemistry of organotin(IV) complexes. Journal of the Iranian Chemical Society, 5, 16–28. http://dx.doi.org/10.1007/BF02245811

Sharma, N., Koitha, A., Bhatt, S. S., & Choudhry, S. C. (2003). Synthesis and characterization of OrganoSn(IV) 2,4-Dinitrophenoxides. Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry, 33, 497–507. http://dx.doi.org/10.1080/089054294080020001

Sharma, P., & Sharma, J. D. (2001). In vitro hemolysis of human erythrocytes—by plant extracts with antiplasmodial activity. Journal of Ethnopharmacology, 74, 239–243. http://dx.doi.org/10.1016/S0378-8741(00)00370-6

Singh, R. V., Fahmi, N., & Bilyo, M. K. (2005). Coordination behavior and biopotency of N and S/O donor ligands with their palladium(II) and platinum(II) complexes. Journal of the Iranian Chemical Society, 2, 40–46. http://dx.doi.org/10.1007/BF02245778

Tabassum, S., & Pettinari, C. (2006). Chemical and biotechnological developments in organotin cancer chemotherapy. Journal of Organometallic Chemistry, 691, 1761–1766. http://dx.doi.org/10.1016/j.jorganchem.2005.12.033

Volla, V., Bakola-Christoupolous, M., Akvivos, P., Kojic, V., & Bogdanovic, G. (2006). Synthesis, structure and in vitro biological activity of new hydroxy-naphthoquinonato triorganotin compounds. Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry, 36, 765–775. http://dx.doi.org/10.1080/153223170601028298

Wark, T. A., & Stephan, D. W. (1990). Earlylate heterobimetallic complexes: Spectrophotometric and spectroscopic and structural studies of thioloato-bridged titanium/copper and vanadium/copper complexes. Inorganic Chemistry, 29, 1731–1736. http://dx.doi.org/10.1021/ic00034a028

Zhang, Y., Wang, X., & Ding, L. (2011). Synthesis and DNA binding studies of Mg(II) complex of Schiff base derived from vanillin and l-tryptophan. Nucleosides, Nucleotides and Nucleic Acids, 30, 49–62. http://dx.doi.org/10.1080/15257770.2010.543117
