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Synthesis and characterization of hetero-bimetallic complexes with 2-mercapto-5-methyl-benzimidazole: theoretical study and biological activities

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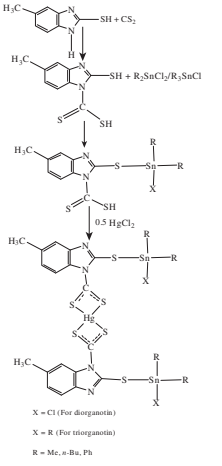

Heterobimetallic complexes have been synthesized by stirring 2-mercapto-5-methylbenzimidazole with carbon disulfide in methanol at room temperature. In the second step, the product was treated with R_2SnCl_2/R_3SnCl (R = Me, *n*-Bu, Ph) in 1 : 1 M ratio, then organotin(IV) complexes were treated with $HgCl_2$ in 2 : 1 M : L ratio to yield heterobimetallic complexes. The ligand and complexes have been characterized by elemental analysis, IR, ^{1}H- and ^{13}C NMR spectroscopy, mass spectrometry (EI-MS), and semiempirical study to assess the binding mode of the heterobimetallic complexes. IR data showed the bidentate nature of the dithiocarbamate moiety, which is also confirmed by semiempirical study. Mass spectra correspond to the expected for the complexes. NMR spectroscopy confirmed the four-coordinate geometry in solution. Computed molecular descriptors, thermodynamic parameters, and electrostatic surface potential map of **4** were calculated by using the PM6 method. Biological screening data indicated that complexes exhibit significant activity against various bacterial and fungal strains with few exceptions. Ligand and **6** are excellent for phagocyte reactive oxygen species inhibition.

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Keywords: 2-Mercapto-5-methyl-benzimidazole; Sn(IV); Hg(II); Spectroscopy; Theoretical study; Biological activities

1. Introduction

Heterobimetallic complexes and networks attract interest owing to the presence of two different metals which contributes to fascinating structures and results in important applications. It seemed appropriate to choose such ligand system for the synthesis of heterobimetallic complexes involving transition metal ions. It has been observed that heterobimetallic complexes contain both electron-deficient and electron-rich transition metal ions behaving in a cooperative manner leading to enhanced reactivity. These systems are of interest because of their structural diversities, molecular magnetism, electrical conductivity and as complex initiators for photooxidation reactions [1–3]. In heterobimetallic complexes, each metal unit can impart, to the resulting macrocycle, a specific property like the capability to absorb or emit visible light and to reversibly exchange electrons. Heterobimetallic complexes have found applications in molecular electronics and information processing [4]. From a biochemical point of view, the presence of two metal ions in close proximity mimic the active site of several metallo-proteins and metallo-enzymes offering an opportunity to study the metal–protein interactions *in vitro*. Heterobimetallic complexes can, therefore, be used as biological models and probes for studying metal–proteins and metal–enzyme interactions. These materials have applications in selective gas sorption, sensing, and catalysis [5, 6]. Identification and recognition of several metal complexes as new anticancer and antimicrobial agents has been related to their structural diversity and the properties arising from binding/interaction with bimolecular cellular targets.

The organotin complexes have been of interest for many years because of their versatile bonding modes. For the development of new drugs, organotin complexes have attained an important place in pharmaceutical and medicinal chemistry [7, 8]. The coordination chemistry of tin is extensive with various geometries and coordination numbers known for both inorganic and organometallic complexes. Organotin(IV) compounds are very important in cancer chemotherapy because of their apoptotic inducing character [9, 10].

Mercury compounds can easily be absorbed by inhalation and through the skin, causing dangerous health problems. The toxicity of mercury salts and inorganic mercury compounds or species is a major reason for renal failure, sudden life-threatening, profound circulatory collapse with tachycardia, hypotension and peripheral vasoconstriction, vomiting, and bloody diarrhea. The brain is mainly the critical organ for chronic inorganic mercury poisoning. Regardless of the form of mercury causing toxicity, chelation therapy should be started when mercury poisoning is suspected in critically ill patients [11]. The synthesis and study of mercury(II) chelates is considered a major discipline in the impact processes of chelation therapy.

Dithiocarbamates are ubiquitous sulfur donor ligands capable of binding nearly all metal ions in various modes, monodentate, terminal bidentate, and bridging bidentate, which allows their complexes to be structurally organized [12]. The dithiocarbamate moiety has been exploited as a useful structural motif for metal directed self-assembled systems having a range of structures like nano-sized resorcarene-based assemblies [13], catenanes [14], assorted macrocycles [15] and cryptands [16]. The wide biological

activity of dithiocarbamate systems may be attributed to their ability to fit into a receptor site and concurrently undergo reversible redox reactions [17]. 2-Mercapto-5-methyl-benzimidazole ligands are of considerable interest as strong chelating compounds toward metal ions [18–24]. We report here the synthesis, characterization, semiempirical study, and biological activities of heterobimetallic complexes containing tin(IV) and mercury(II) with 2-mercapto-5-methyl-benzimidazole.

2. Experimental

2.1. *Chemicals and instrumentation*

2-Mercapto-5-methyl-benzimidazole, carbon disulfide, dimethyltin dichloride, trimethyltin chloride, dibutyltin dichloride, tributyltin chloride, diphenyltin dichloride, triphenyltin chloride, methanol, and DMSO were purchased from Sigma Aldrich Chemical Company (USA). Chloroform was purchased from Merck (USA). Nutrient agar and potato dextrose agar were purchased from Oxoid Company (UK). All reagents and solvents were purchased commercially and used without any further purification. Solvents were dried by standard procedures [25].

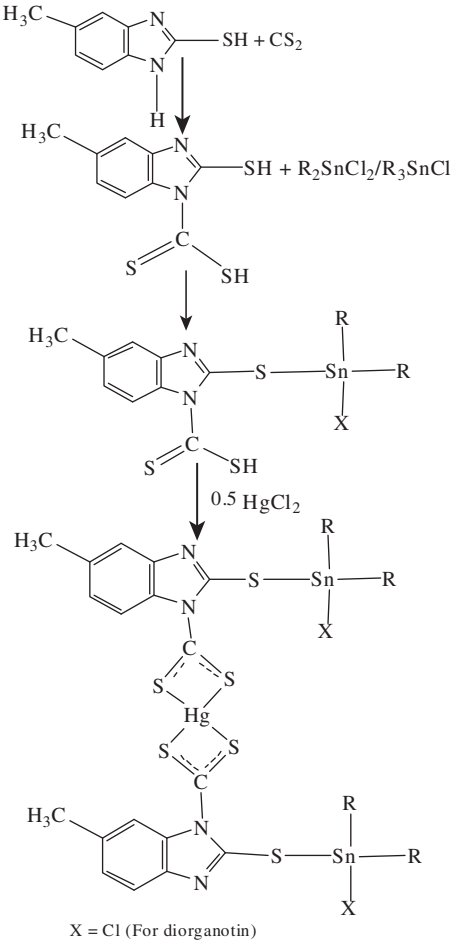
Samples were taken in capillary tubes and their melting points were determined using an electrothermal melting point apparatus, Model Stuart (SMP3) UK and are uncorrected. Elemental analyses (carbon, hydrogen, nitrogen, and sulfur) were done by a CHNS-932 elemental analyzer Leco Corporation (USA). Infrared absorption spectra were recorded as KBr/CsBr discs on a Perkin Elmer-1000 spectrophotometer from 4000 to 250 cm^{-1}. NMR spectra were recorded on a Bruker ARX 300 MHz FT-NMR spectrometer using $CDCl_3$ as internal reference. EI-MS spectra were recorded on a JEOL JMS 600-H mass spectrometer. The antimicrobial activities of the ligand and complexes were observed in an incubator (Sanyo, Germany) and sterilized in an autoclave (Omron, Japan). The Luminometer used in oxidative burst assay was a Luminoskan EL, RT, RS (Helsinki, Finland). Semiempirical calculations were done by MOPAC 2007 [26] in gas phase by using the PM6 method [27].

2.2. *General procedure for the synthesis of heterobimetallic complexes 1–6*

Step I. 2-Mercapto-5-methyl-benzimidazole (1 mmol) was dissolved in methanol (50 mL) in a two-necked round-bottom flask (250 mL) with continuous stirring, and then, carbon disulfide (1 mmol) was added dropwise in the above solution with constant stirring at room temperature. The reaction mixture was continuously stirred for 30 min at room temperature.

Step II. To the above solution, diorganotin dichloride or triorganotin chloride (1 mmol) was added as solid in portions and the reaction mixture was further stirred for 3 h at room temperature.

Step III. Then, mercuric chloride (0.5 mmol) was added as solid in portions with constant stirring in the above reaction mixture. Reaction mixture was stirred for further 3 h at room temperature. Precipitates obtained were filtered off and dried in open air. Product obtained was recrystallized from chloroform : petroleum ether (1 : 1).



X = Cl (For diorganotin)

X = R (For triorganotin)

R = Me, *n*-Bu, Ph

R	Me	*n-Bu*	Ph
Compound	**1, 4**	**2, 5**	**3, 6**

2.3. *Antimicrobial activity*

2.3.1. Bacterial growth medium, cultures, and inoculum preparation. Pure cultures were maintained on nutrient agar medium in the slants and petri plates. For the inoculums preparation, 13 g L^{-1} of nutrient broth was suspended in distilled water, mixed well and distributed homogenously and autoclaved. 10 µL of pure culture of a bacterial strain was mixed in medium and placed in a shaker for 24 h at 37 °C. The inocula were stored at 4 °C. The inocula with 1×10^8 spores mL^{-1} were used for further analysis.

2.3.2. Fungal growth medium, culture, and inoculum preparation. Pure culture of the fungi were maintained on sabouraud dextrose agar medium (65 g L^{-1}) in slant and petri plates that were placed in hot air oven at 180 °C for 3 h under presterilized conditions. These culture slants were incubated at 28 °C for 3–4 days for the multiplication of fungal strains.

2.3.3. Antimicrobial assay by disc diffusion method. The antimicrobial activities of the compounds were screened by using the disc diffusion method [28]. For bacterial and fungal strains assays, nutrient agar (28 g L^{-1}) and potato dextrose agar (39 g L^{-1}), respectively, were dissolved in distilled water. The medium was autoclaved at 121 °C for 15 min and used to culture bacteria. Before the medium was transferred to petri plates, inoculums (100 µL/100 mL) were added to the medium and poured in sterilized petri plates. After this, small filter paper discs were laid flat on growth medium containing 100 µL of sample. The petri plates were then incubated at 37 °C for 24 h and at 28 °C for 48 h for the growth of bacteria and fungi, respectively. The sample having antimicrobial activity exhibited clear zones around the discs. The zones of inhibition were measured in millimeters using a zone reader [29]. Triplicate petri plates were prepared. Rifampicin and fluconazol (20 µg $disc^{-1}$) were used as positive control for bacteria and fungi, respectively.

2.4. *Immunomodulatory activity*

Luminal enhanced chemiluminescence assay was carried out by using the Helfand and Haklar method [30, 31]. Precisely, 25 µL of serially diluted compounds with concentration range between 1 and 100 mg mL^{-1} was incubated with 25 µL diluted whole blood in 1 : 50 dilution in sterile $HBSS^{++}$ ($HBSS^{++}$ = Hank's balanced salt solution with Ca^{+2} and Mg^{+2} ions). These tests were carried out in white 96-well plates which were incubated at 37 °C for 30 min in the thermostated chamber of the luminometer. $HBSS^{++}$ and cells without compounds were used as negative and positive control, respectively. After 15 min of incubation of whole blood Opsonized zymosan-A, 25 µL, followed by 25 µL luminol (7×10^{-5} M) along with $HBSS^{++}$ was added to each well to get a volume of 100 mL of each well. The luminometer results were monitored as chemiluminescence relative high unit (RLU) with peak and total integral values set for 50 min repeated scans at 30-s intervals and 1 s points measuring time. The total ROS level was recorded as total light produced during the 50 min scan. The percentage of inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = 100 - (\text{RLU of test sample}/\text{RLU of the control}) \times 100$$

2.5. *Statistical analysis*

Antimicrobial assay values are presented as means ± SD of each measurement. The data were checked by one-way ANOVA using Minitab 15. Pearson correlation coefficients and p-values were applied to exhibit correlations and their significance. Differences of $p < 0.05$ were considered substantive [32].

Immunomodulatory activity results were processed by using Soft Max Pro 4.8 software (Molecular Devices, CA, USA) and then by MS Excel. Results were presented as means ± standard error mean from triplicate ($n = 3$) observation. IC_{50} values were determined by using EZ-FIT, Enzyme kinetics software by Perrella Scientific, Inc. USA.

2.6. *Semiempirical study*

The semiempirical calculations were done by MOPAC 2007 [26] program in gas phase using the PM6 method [27]. The model was built by using constraints on the movement of each of the four coordinating sulfurs, carbons connected to sulfur and on the Hg^{+2} ion on the x, y, z axes and performing the geometry optimization. The constraints on carbons bonded to S were removed first, followed by those on the sulfurs one by one and finally on Hg^{+2}. The final geometry optimization was done without constraints on any of the atoms. The final RMS values were always less than one. There were no imaginary frequencies in the vibrational analysis.

3. Results and discussion

All newly synthesized complexes are solid and stable in air. They have sharp melting points and are soluble in common organic solvents. The elemental analysis data show that observed values are in agreement with the calculated values. The physical data are given in table 1.

3.1. *Infrared spectroscopy*

The IR spectroscopy is one of the most frequently employed techniques for the characterization of the tin(IV) and mercury(II) compounds. The infrared spectra of **HL** and

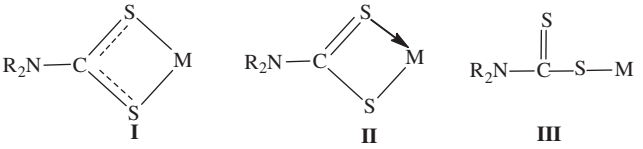
Table 1. Physical data of heterobimetallic complexes containing tin(IV) and mercury(II).

Compound	Molecular formula	Molecular weight	M.P (°C)	Yield (%)	Elemental analysis calculated (found)			
					%C	%H	%N	%S
HL	$C_8H_8N_2S$	164.23	290–293	–	58.51 (58.45)	4.91 (4.95)	17.06 (17.11)	19.52 (19.48)
1	$C_{22}H_{24}Cl_2HgN_4S_6Sn_2$	1045.77	270–272	77	25.27 (25.30)	2.31 (2.35)	5.36 (5.66)	18.40 (18.44)
2	$C_{34}H_{48}Cl_2HgN_4S_6Sn_2$	1214.08	260–263	71	33.64 (33.68)	3.99 (3.96)	4.61 (4.65)	15.84 (15.89)
3	$C_{42}H_{32}Cl_2HgN_4S_6Sn_2$	1294.04	236–238	67	38.99 (38.93)	2.49 (2.53)	4.33 (4.28)	14.87 (14.92)
4	$C_{24}H_{30}HgN_4S_6Sn_2$	1004.94	258–259	64	28.69 (28.64)	3.01 (3.06)	5.58 (5.53)	19.14 (19.11)
5	$C_{42}H_{66}HgN_4S_6Sn_2$	1257.41	241–243	80	40.12 (40.08)	5.29 (5.33)	4.46 (4.49)	15.30 (15.26)
6	$C_{52}H_{42}HgN_4S_6Sn_2$	1377.34	173–174	73	46.15 (45.30)	3.13 (3.11)	4.14 (4.03)	14.21 (13.93)

Table 2. IR data (cm^{-1}) of heterobimetallic complexes containing tin(IV) and mercury(II).

Compound	ν(N–H)	ν(C–N)	ν(S–H)	ν(C–S)	ν(C=S)	ν(Sn–C)	ν(Sn–S)	ν(Sn–Cl)	ν(Hg–S)
HL	3120	1473	2682	848	1037	–	–	–	–
1	–	1456	–	989	1070	589	425	316	369
2		1461	–	926	1077	589	425	305	396
3	–	1456	–	936	1060	289	420	315	350
4	–	1451	–	979	1065	569	425	–	368
5	–	1450	–	925	1060	589	425	–	396
6	–	1456	–	995	1074	280	436	–	351

complexes were recorded as KBr/CsBr pellets from 4000 to 250 cm^{-1}. The IR spectrum of the free ligand was compared with the spectra of complexes to study the binding of dithiocarbamate to tin(IV) and mercury(II) in the new complexes. Several significant changes with respect to the ligand were observed in the reported complexes, which are listed in table 2. Dithiocarbamates coordinate to metals in three different ways (**I-III**), bidentate(I), anisobidentate(II), or monodentate(III) [33].



The complexation has been confirmed by the disappearance of ν(N–H) and ν(S–H) at 3120 and 2682 cm^{-1}, respectively, in spectra of the complexes [34–36]. A strong band at 1461–1450 cm^{-1} is attributed to ν(C–N) in the complexes [37, 38]. In **1–6**, ν(C–S) and ν(C=S) bands are 1077–1060 cm^{-1} and 995–925 cm^{-1}, respectively. Appearance of a strong band at 1000 ± 20 cm^{-1} suggests bidentate chelating mode of the dithiocarbamate with dithiocarbamate symmetrically coordinated to the metal ions [39].

The appearance of ν(Sn–C), ν(Sn–S), and ν(Sn–Cl) stretches at 589–569, 436–420 and 316–305 cm^{-1}, respectively, confirmed the complexation. Stretching vibration of Sn–C appeared at 289 and 280 cm^{-1} in di- and triphenyltin(IV) complexes, respectively [40–43]. A distinct medium to sharp band at 396–350 cm^{-1} attributed to ν(Hg–S) further confirms the complexation [44, 45].

3.2. *^{1}H NMR spectroscopy*

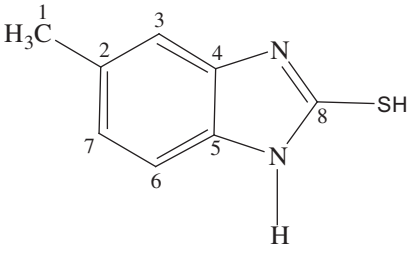
The characteristic signals in the ^{1}H NMR spectra of the ligand and complexes, recorded in d_6-DMSO, are given in table 3. All protons present in the ligand and synthesized complexes **1–6** have been identified in position and number with the protons calculated from incremental method [46]. The deprotonation of the ligand during complexation has been confirmed by the absence of –NH and –SH signals at 10.52 and 12.37 ppm, respectively [34–38].

The methyl group attached to tin(IV) gives a sharp singlet at 0.98 and 1.25 ppm in **1** and **4** with $^2J[^{119}Sn,^1H]$ of 83 and 81 Hz and $^3J[^1H,^1H]$ of 7.2 Hz [47, 48]. Four sets of signals were observed for the protons of *n*-butyl attached to tin(IV) in **2** and **5** at 1.27–1.66 and 1.11–1.61 ppm as multiplets and at 0.87 and 0.89 ppm as triplets with $^3J[^1H,^1H]$ of 7.2 Hz [47]. In **3** and **6**, the appearance of the multiplets at 6.82–6.95 and 7.36–7.52 ppm,

Table 3. ^{1}H NMR data[a–d] (ppm) of heterobimetallic complexes containing tin(IV) and mercury(II).

Proton no.	HL	1	2	3	4	5	6
1	2.33s	2.35s	2.36s	2.35s	2.35s	2.36s	2.36s
3	6.94s	7.04s	7.08s	7.02s	7.05s	7.05s	7.04s
6	6.91d (7.3)	7.08d (7.2)	7.02d (7.3)	7.07d (7.2)	7.06d (7.3)	7.11d (7.2)	7.06d (7.3)
7	7.00d (7.3)	7.17 (7.2)	7.16d (7.3)	7.15 (7.2)	7.17d (7.3)	7.20d (7.2)	7.14d (7.3)
–SH	12.37s	–	–	–	–	–	–
–NH	10.52s	–	–	–	–	–	–

[a]Compound **1**: Sn–CH_3, 0.98s, ^{2}J[83]; **2**: Sn–$CH_2CH_2CH_2CH_3$, 1.27–1.66 m, 0.87t (7.2); **3**: Sn–C_6H_5, 6.82–6.95 m; **4**: Sn–CH_3, 1.25s, ^{2}J[81]; **5**: Sn–$CH_2CH_2CH_2CH_3$, 1.11–1.61 m, 0.89t (7.2); **6**: Sn–C_6H_5, 7.36–7.52 m.
[b]Chemical shifts (δ) in ppm. ^{2}J[^{119}Sn,^{1}H] and ^{3}J(^{1}H,^{1}H) in Hz are listed in square brackets and parenthesis, respectively.
[c]Multiplicity is given as: s – singlet, d – doublet, t – triplet, m – multiplet.
[d]



respectively, was ascribed to protons of phenyl attached to tin(IV) and ^{3}J[^{1}H,^{1}H] of 7.2 Hz [49–51].

3.3. *^{13}C NMR spectroscopy*

^{13}C NMR spectra of the ligand and complexes were recorded in d_6-DMSO and data are given in table 4. Complexation of the ligand with tin(IV) and mercury(II) has been confirmed by the presence of sharp signals of C-8 and -CSS from 158.15–159.93 ppm to 164.14–162.80 ppm in the spectra of complexes. All the carbons present in the ligand and complexes (**1–6**) have been identified at similar position and number with the carbons calculated from incremental method [46]. The information about the possible coordination

Table 4. ^{13}C NMR data[a,b] (ppm) of heterobimetallic complexes containing tin(IV) and mercury(II).

Carbon no.	HL	1	2	3	4	5	6
1	21.42	20.96	20.95	20.93	20.94	20.97	20.93
2	130.68	129.71	130.56	129.76	129.97	130.51	129.09
3	109.59	110.73	110.56	110.36	110.49	111.23	110.08
4	132.95	133.44	132.77	133.97	133.08	133.32	134.66
5	132.09	131.92	132.72	131.99	132.18	132.64	130.09
6	123.65	124.89	124.31	124.53	124.55	124.83	123.12
7	110.07	110.93	110.81	110.63	110.74	111.23	110.40
8	159.16	159.93	159.85	158.15	159.32	158.51	158.21
–CSS	–	161.32	161.15	161.80	161.14	161.95	162.80

[a]Compound **1**: Sn–CH_3, (C-α) 10.05 ^{2}J[392]; **2**: Sn–$CH_2CH_2CH_2CH_3$, (C-α) 27.56 ^{1}J[341], (C-β) 27.26 ^{2}J[20], (C-γ) 25.46 ^{3}J[62], (C-δ) 13.65; **3**: Sn–C_6H_5, (C-α) 133.08 ^{1}J[645], (C-β) 132.18 ^{2}J[45], (C-γ) 128.35, (C-δ) 124.55; **4**: Sn–CH_3, (C-α) 10.06 ^{2}J[392]; **5**: Sn–$CH_2CH_2CH_2CH_3$, (C-α) 27.67 ^{1}J[341], (C-β) 27.52 ^{2}J[20], (C-γ) 25.50 ^{3}J[62], (C-δ) 13.72; **6**: Sn–C_6H_5, (C-α) 132.61 ^{1}J[645], (C-β) 132.31 ^{2}J[45], (C-γ) 128.36, (C-δ) 124.12.
[b]Chemical shifts (δ) in ppm. ^{n}J[^{119}Sn,^{13}C] in Hz is listed in square brackets.

geometries in solution was obtained from $^{1}J[^{119}Sn–^{13}C]$ and $^{2}J[^{119}Sn–^{1}H]$ coupling constants [52–54]. The $^{1}J[^{119}Sn–^{13}C]$ in the complexes suggests the tin is four coordinate in solution [55].

3.4. *Mass spectrometry*

Mass spectra were recorded at 70 eV for ligand and **2**, **4**, and **6**. In **2**, **4**, and **6**, molecular ion peak was not observed. It appears that bond dissociation energies are relatively low so that they suffer considerable fragmentation. In the ligand, the base peak is the molecular ion peak due to $[C_8H_9N_2S]^{\cdot+}$ at *m/z* 164(100), while in **2**, **4**, and **6** the base peak is due to $[C_8H_9N_2S]^{+}$, $[Sn(CH_3)_3]^{+}$, and $[SSnC(C_6H_5)_2]^{+}$ at *m/z* 163(100), 164(100), and 308(100), respectively.

In the ligand and **2**, **4**, and **6**, primary fragmentation was due to successive loss of –SH and $–CH_3$ groups followed by the disintegration of the $[C_6H_5]^{+}$, $[C_5H_4]^{+}$ and formation of $[C_4H_3]^{+}$ as the end product which is in accord with earlier reports [56, 57]. In the second step for **2**, **4**, and **6**, fragmentation involves formation of $[Hg]^{+}$ with successive elimination of CS_2. In these complexes, another possible route involves formation of the alkyltin(IV) complexes with stepwise elimination of R groups to $[Sn]^{+}$ and $[SnH]^{+}$ as the end product.

3.5. *Semiempirical study*

In **4**, Hg is surrounded by four sulfurs of dithiocarbamate to form the square-planar seven-membered cyclic core (HgS_4C_2) with an appropriate square-planar chelating conformation defined by S–C–S–Hg–S–C–S.

Hg^{+2} lies in the plane formed by the four sulfurs in a square-planar arrangement (0.01 Å away from the mean SSSS plane). In **4**, the calculated bond angles and bond lengths are all typical of organotin compounds [58]. However, Hg^{+2}–S bond lengths of 2.41–2.73 Å have been observed in the dithiocarbamate complexes of Hg^{+2} [59]. The values are shorter than the sum of van der Waals radii for these ions 3.68 Å [60].

Orbital calculations provide a detailed description of the orbitals, including spatial characteristics, nodal patterns, and individual atomic contributions [61]. HOMO, LUMO, charge distribution, and electrostatic surface potential map of **4** are given in figures 1–3.

3.6. *Antibacterial activity*

The antibacterial activity was recorded by the disc diffusion method [28]. The results are summarized in table 5. **HL** shows no activity against *S. aureus* and *P. multocida*, while exhibit moderate activity against *B. subtilis* and *E. coli* with zone diameter 13.8 and 17.1 mm, respectively. It indicates that *S. aureus* and *P. multocida* are resistant toward ligand [41].

The heterobimetallic complexes have increased antibacterial activity due to increased toxicity against bacteria, in accord with earlier reports [62, 63]. By comparing the results of **1–6** with mono- and homobimetallic complexes [55, 64, 65], it might be concluded that these complexes have greater inhibiting power against all the microbes. The enhanced antibacterial activity may be due to the presence of Hg(II) along with Sn(IV). The results are also compared with reference drug (rifampicin) which shows that the synthesized complexes are active but their activity was lower than the standard drug. Complex **2** exhibits

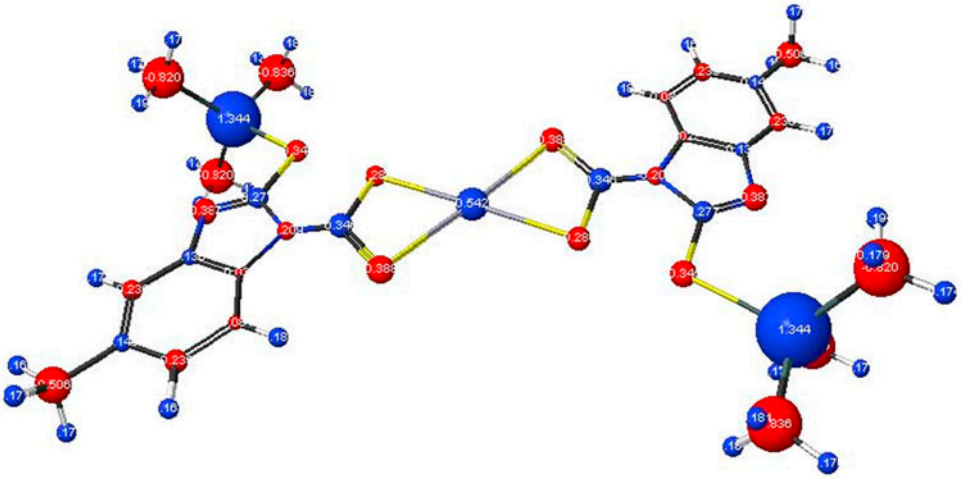


Figure 1. Charge distribution of **4**.

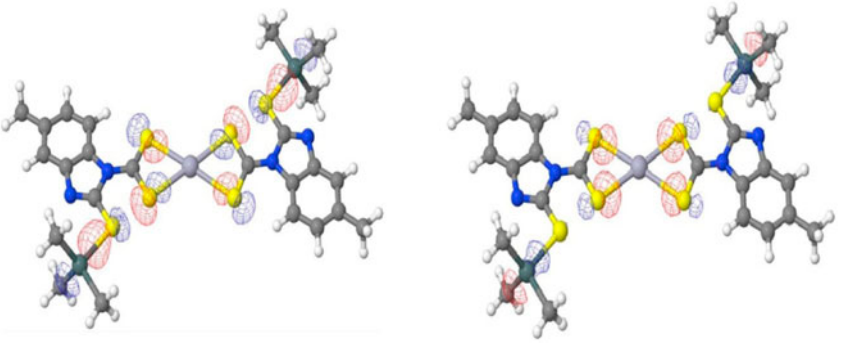


Figure 2. HOMO and LUMO of heterobimetallic **4**.

significant antibacterial activity against *B. substilis*, *S. aureus*, *E. coli,* and *P. multocida*, while **1**, **3**, **4**, **5**, and **6** exhibit moderate activity against all bacterial strains. The presence of methyl in **1** and **4** increases the lipophilic character of the ligand and this increased lipophilicity facilitates the penetration of the complexes into the lipid membranes, further restricting proliferation of the microorganisms [63]. That complexes show better antibacterial activity than free ligand means that complexes can be used as potential bactericides.

3.7. *Antifungal activity*

The antifungal activities of **HL** and **1–6** against four fungal strains, *A. niger*, *A. flavus*, *A. alternata,* and *H. myedis*, are checked by using disc diffusion method [28]. The results are compared with standard drug fluconazol, used as positive control and are given in table 6.

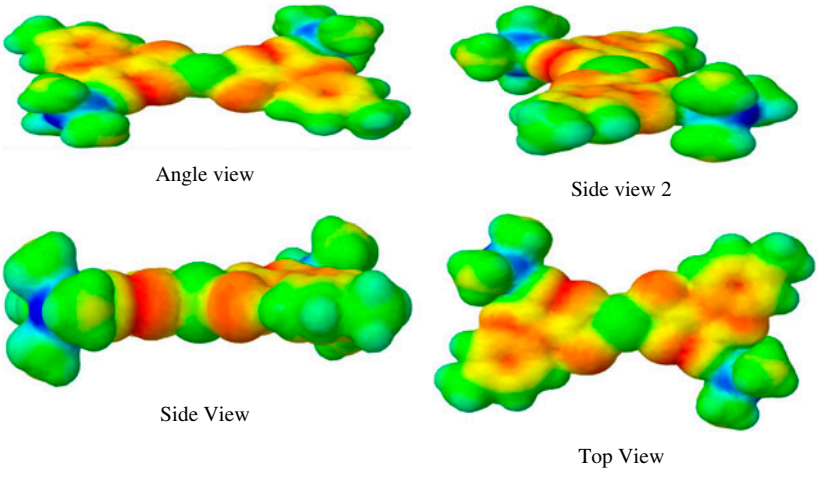


Figure 3. Electrostatic surface potential map of **4**.

Table 5. Antibacterial activity of heterobimetallic complexes containing tin(IV) and mercury(II)[a,b].

	Bacterial zone size (mm)			
Compound	*B. subtilis*	*S. aureus*	*E. coli*	*P. multocida*
HL	13.8[b] ± 0.7	0.0[c] ± 0.0	17.1[b] ± 0.1	0.0[c] ± 0.0
1	16.0[b] ± 0.4	16.0[b] ± 0.5	18.0[b] ± 0.6	17.0[b] ± 0.3
2	23.0[a] ± 0.6	22.0[a] ± 0.6	25.0[a] ± 0.5	26.0[a] ± 0.7
3	16.0[b] ± 0.4	16.0[b] ± 0.7	17.0[b] ± 0.6	18.0[b] ± 0.6
4	15.0[b] ± 0.3	16.0[b] ± 0.4	17.0[b] ± 0.2	16.0[b] ± 0.4
5	16.0[b] ± 0.3	15.0[b] ± 0.3	16.0[b] ± 0.4	17.0[b] ± 0.4
6	15.0[b] ± 0.2	14.0[b] ± 0.5	15.0[b] ± 0.3	16.0[b] ± 0.3
Rifampicin	32.0[a] ± 0.7	30.0[a] ± 0.5	36.0[a] ± 0.5	40.0[a] ± 0.5

[a]Values are mean ± SD of three samples analyzed individually in triplicate at $p < 0.05$.
[b]0–5 – no activity, 5–10 – activity present, 10–20 – moderate activity, 20–30 – strong activity.
[c]Lowest activity.

Table 6. Antifungal activity of heterobimetallic complexes containing tin(IV) and mercury(II)[a,b].

	Fungal zone size (mm)			
Compound	*A. niger*	*A. flavus*	*H. myedis*	*A. alternate*
HL	0.0[c] ± 0.0	0.0[c] ± 0.0	–	0.0[c] ± 0.0
1	15.0[b] ± 0.4	16.0[b] ± 0.5	19.0[b] ± 0.6	16.0[b] ± 0.5
2	22.0[a] ± 0.7	20.0[a] ± 0.6	24.0[a] ± 0.8	25.0[a] ± 0.7
3	16.0[b] ± 0.5	17.0[b] ± 0.4	13.0[b] ± 0.3	15.0[b] ± 0.4
4	15.0[b] ± 0.4	15.0[b] ± 0.3	16.0[b] ± 0.4	16.0[b] ± 0.5
5	14.0[b] ± 0.3	14.0[b] ± 0.4	15.0[b] ± 0.5	16.0[b] ± 0.5
6	14.0[b] ± 0.3	14.0[b] ± 0.3	17.0[b] ± 0.6	14.0[b] ± 0.3
Fluconazol	28.0[a] ± 1.1	28.0[a] ± 0.5	26.0[a] ± 0.5	26.5[a] ± 0.5

[a]Values are mean ± SD of three samples analyzed individually in triplicate at $p < 0.05$.
[b]0–5 – no activity, 5–10 – activity present, 10–20 – moderate activity, 20–30 – strong activity.
[c]Lowest activity.

Complex **2** shows strong activity against all fungal strains. Complexes **1** and **4–6** indicate moderate activity against *A. niger*, *A. flavus*, *H. myedis,* and *A. alternata*. The complexes are more active against fungal strains than ligand due to the presence of sulfur in complexes and attachment of tin(IV) and mercury(II) [66]. The increased activity of the complexes is due to the effect of metal on the normal cell process; chelation increases the lipophilic character of the metal. This favors its permeation through the lipid layer of the membrane, thereby resulting in interference with normal cell process [67].

From the data, it might be concluded that heterobimetallic compounds are more effective against the fungi in contrast to earlier reported compounds [55, 64, 65]. This might be due to the presence of Hg(II) along with Sn(IV) which enhances the fungicidal activities of the complexes.

3.8. *Structure–activity relationship*

Metal ions are adsorbed on the cell walls of microorganisms, disturbing the respiration processes of the cells, thus blocking the protein synthesis that is required for further growth of the organisms. Hence, metal ions are essential for the growth-inhibitory effects [68].

Increased lipophilicity facilitates the penetration of the complexes into lipid membrane [69, 70], restricting proliferation of the microorganisms. The variations in the effectiveness of different compounds against different organisms depend either on the impermeability of the microbial cells or on differences in the ribosome of the cells. The mode of action of antimicrobials may involve various targets in the microorganisms. These targets include the following:

(1) The higher activity of the metal complexes may be due to the different properties of the metal ions upon chelation. The polarity of the metal ions will be reduced due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with donor groups. Thus, chelation enhances the penetration of the complexes into lipid membranes and the blockage of metal-binding sites in the enzymes of the microorganisms [71–73].
(2) Deactivation of various cellular enzymes plays a vital role in the metabolic pathways of these microorganisms.
(3) Denaturation of one or more cellular proteins, causing the normal cellular processes to be impaired.
(4) Formation of hydrogen bond through the –NH and –SH groups with the active center of various cellular constituents, resulting in interference with normal cellular processes [74].
(5) These complexes may also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism [75].
(6) The availability of coordination positions at metal.

3.9. *Immunomodulatory activity*

HL, **2**, **4**, and **6** are tested for their possible immunomodulatory activity using Luminol-enhanced chemiluminescence assay [30, 31]. The effect of the test compounds on whole blood phagocytes indicates that **HL** and **6** show potent inhibitory activity against ROS inhibition with IC_{50} values of $9.1 \pm 0.8\ \mu g\ mL^{-1}$ and $<1\ \mu g\ mL^{-1}$, respectively, as

compared to the standard, ibuprofen (11.8 ± 1.9 μg mL^{-1}). Complexes **2** and **4** do not exhibit ROS activity even at very high IC_{50} values (>100 μg mL^{-1}) (Supplementary Material). The results of chemiluminescence assay and structures of these compounds suggest that the presence of amide analogs with varying substituted exocyclic phenyl rings cause potent immunosuppressive activity. This is due to the fact that amide group has an ability to be protonated at physiological (pH 7.4) to form ionic, hydrogen, dipole–ionic, or dipole–dipole bonding with target molecules which strengthen the pharmacodynamic properties [76]. The stability of these amide analogs with varying substituted exocyclic phenyl rings and supplementary binding sites in drug molecule is important in the immunosuppressive activity via neutralization of harmful radicals [77, 78].

The higher activity of **6** containing triphenyltin(IV) may be due to the formation and transportation of $Ph_3Sn(IV)^+$ across the cellular membrane as part of the mechanism inhibition. This is probably due to the presence of three phenyl groups around tin and lower number of coordinating sites/bonds which facilitate the easier formation of $Ph_3Sn^+(IV)$.

The lower activity of *n*-$Bu_2Sn(IV)$ complex, **2**, is probably due to butyl groups, which would increase the electron density around tin forming relatively more stable Sn–C bonds upon complexation, thereby diminishing the easy delivery of Bu_2Sn^{2+} [79, 80].

4. Conclusion

IR data revealed that the ligand is bidentate and the complexes four-coordinate around mercury(II) and tin(IV), which is also confirmed by semiempirical study. NMR showed four-coordinate geometry in solution. Positive heat of formation showed that **4** is thermodynamically unstable and chemically labile. The antimicrobial assay of ligand and complexes against different bacterial and fungal strains showed that all heterobimetallic complexes are more active as compared to free ligand and mono/homobimetallic complexes. Immunomodulatory activity exhibited that ligand and **6** are potent immunomodulating agents, which may act as reactive oxygen species inhibitors.

Supplementary material

Selected bond lengths (Å) and angles (°) of **4** (table S1); computed molecular descriptors of heterobimetallic **4** (table S2); computed thermodynamic parameters of heterobimetallic **4** (table S3); immunomodulatory activity data of **HL** and **2**, **4** and **6** (table S4).

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed here [http://dx.doi.org/10.1080/00958972.2015.1042374].

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