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Complexations of 2-thiouracil and 2,4-dithiouracil with $Cd(SeCN)_2$ and $Hg(SeCN)_2$: NMR and anti-bacterial activity studies

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Abstract. Cadmium and mercury selenocyanate complexes of 2-thiouracil (TU) and 2,4-dithiouracil (DTU) ligands have been synthesized to form complexes of the type $[M(SeCN)_2(TU)]$ and $[M(SeCN)_2(DTU)]$ (where M is Cd^{2+} or Hg^{2+}) and studied by various spectroscopic techniques such as IR, ^1H and ^{13}C NMR in solution and in the solid state for ^{13}C, ^{15}N, and ^{113}Cd nuclei. Based on IR, and solution and solid-state ^{13}C NMR data, stronger cadmium bonding to the thiouracil was observed compared to that of mercury. Anti-bacterial activities of these complexes have been investigated with standard type culture of *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella typhi* (MTCC 733) and *Staphylococcus aureus* (MTCC 737) and show that ligands exhibit more anti-bacterial activities than that of the corresponding Cd(II) and Hg(II) complexes.

Keywords: Cadmium(II) complexes, mercury(II) complexes, thiouracil ligands, solid-state NMR, anti-bacterial activities

1. Introduction

The coordination chemistry of nucleobases, such as thiouracil and 2,4-dithiouracil, to metal ions has been an active area of research during past decades [5,11,19]. Most of these studies were dedicated especially to biologically relevant metals in their positive oxidation states, such as Mg^{2+}, Cd^{2+}, Hg^{2+} etc. [6]. Generally these areas of research have been directed towards the examination of the coordination modes of neutral nucleobases to metals. But complexes of heterocyclic ligands with metal ions, particularly Cd(II) and Hg(II) are of special interest in bioinorganic chemistry because of concerns regarding their impact on the environment and its toxicological effects in marine microorganisms [2,8,16, 17]. The cadmium and mercury complexes present a significant advantage over their zinc counter parts from the structural characterization point of view, since ^{113}Cd and ^{199}Hg NMR spectroscopy provides deeper insight of the coordination sphere of the complexes [4,21,22].

The coordination behavior of selenocyanate ion is important because of its different binding modes. Since Se has the tendency to coordinate preferably to soft metal like cadmium(II), the $SeCN^-$ acts as a monodentate ligand and this will be ligated through Se. It is also worth noting that, according to

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Jorgenson symbiotic theory [9], hard species will tend to increase the hardness of the atom to which they are bound to. Conversely, same thing happen in presence of soft atom, like Se, coordinate to metal center making the metal center softer. In this context, it would be interesting to study the coordination chemistry in presence of 2-thiouracil (TU) and 2,4-dithiouracil (DTU) ligand having both soft (C=S) and hard (–NH) donors.

Tiekink and co-workers have shown that thiouracil-containing complexes are effective antitumor and arthritic compounds *in vivo* [1,20]. Here, we have also explored the synthesis and its anti-bacterial properties of the prepared complexes.

2. Experiment

2.1. Chemicals

The ^{15}N label 2,4-dithiouracil ligand was obtained from Cambridge Isotope Labs, USA. All other reagents and solvents used were obtained from Aldrich Chemical Co.

All reactions were carried out under an atmosphere of nitrogen. Elemental analyses were performed on Perkin–Elmer Series 11 (CHNS/O), Analyzer 2400 and data are given in Table 1. The IR spectra of the ligands and their Cd(II) and Hg(II) complexes were performed on a Perkin–Elmer FTIR 180 spectrophotometer using KBr pellets over the range 4000–400 cm^{-1}. The selected IR data are shown in Table 2. The ^{1}H and ^{13}C NMR experiments were performed on a Bruker Advance 400 or Jeol JNM-LA 500 spectrometers. ^{1}H and ^{13}C NMR chemical shifts were given as δ-values with reference to tetramethylsilane (TMS) as an internal standard. The NMR data are shown in Tables 3, 4 and 5. Standard type culture of *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella typhi* (MTCC 733) and *Staphylococcus aureus* (MTCC 737) were obtained from Microbial Type Culture Collection (MTCC) Chandigrah, India. The selected data are shown in Table 6.

2.2. Solid-state NMR studies

Natural abundance ^{13}C, ^{15}N and ^{113}Cd, NMR spectra were obtained on a JEOL LAMBDA 500 spectrometer operating at 125.65, 50.55 and 110.85 MHz, respectively, corresponding to a magnetic field of 11.74 T, at ambient temperature of 25°C. Samples were packed into 6 mm zirconia rotors. Cross polarization and high power decoupling were employed. Pulse delay of 7.0 s and a contact time of 5.0 ms were used for cadmium and carbon observations in the CPMAS experiments, whereas the pulse delay of 10 s and a contact time of 6.0 ms were used in the selenium observation. The magic angle spinning rates were from 3000 to 5000 Hz. The cadmium chemical shifts were referenced using a secondary reference $Cd(NO_3)_2 \cdot 4H_2O$, by settings the peak to -100 ppm relative to 0.1 M $Cd(ClO_4)_2$ with ionic

Table 1
Elemental analysis and of the prepared complexes

Complexes	M. Pt.[a] (°C)	Found (calcd.)%		
		C	H	N
$(TU)Cd(SeCN)_2$	135–138	16.35 (16.00)	0.75 (0.67)	12.77 (12.44)
$(TU)Hg(SeCN)_2$	Decomp. 188	13.45 (13.40)	0.64 (0.56)	10.79 (10.42)
$(DTU)Cd(SeCN)_2$	Decomp. 197	15.70 (15.48)	0.88 (0.65)	12.45 (12.01)
$(DTU)Hg(SeCN)_2$	Decomp. 180	13.30 (13.01)	0.63 (0.55)	10.48 (10.12)

Note: [a] M. Pt. – melting points.

Table 2
IR frequencies, ν (cm^{-1}) of ligands and their complexes

Species	ν(SeCN)	ν(N–H)	ν(C=O) and ν(C=S)
$Hg(SeCN)_2$	2127	–	–
$Cd(SeCN)_2$	2107	–	–
TU	–	3136	1711, 1689, 1570
DTU	–	3444	1611, 1588, 1573
$[(TU)Cd(SeCN)_2]$	2109	3449	1706, 1665, 1564
$[(DTU)Cd(SeCN)_2]$	2108	3447	1653, 1599, 1542
$[(TU)Hg(SeCN)_2]$	2117	3447	1699, 1600, 1561
$[(DTU)Hg(SeCN)_2]$	2111	3445	1600, 1539

Table 3
^{13}C NMR chemical shifts of ligands and their Cd(II) and Hg(II) complexes in DMSO-d_6

Species	SeCN	C-2	C-4	C-5	C-6
$Hg(SeCN)_2$	105.83				
$Cd(SeCN)_2$	116.92				
TU	–	161.23	176.17	142.00	105.50
DTU	–	172.96	187.92	136.80	117.24
$[(TU)Cd(SeCN)_2]$	119.58	165.09	177.53	144.09	107.18
$[(DTU)Cd(SeCN)_2]$	117.30	175.04	187.35	143.63	116.38
$[(TU)Hg(SeCN)_2]$	105.91	161.62	173.26	144.72	106.14
$[(DTU)Hg(SeCN)_2]$	106.41	168.92	186.65	142.83	116.14

Table 4
Solid-state ^{13}C isotropic chemical shifts (δ_{iso}) and principle shielding tensors (σ_{xx})[a] of complexes Cd(II) and Hg(II)-selenocyanate complexes with thiouracil and 2,4-dithiouracil

Complex	Nucleus	δ_{iso}	σ_{11}	σ_{22}	σ_{33}	$\Delta\sigma$	η
$Cd(SeCN)_2$	^{113}Cd	211.9	322	283	30	291	0.73
	^{77}Se	−119.6	53	41	−452	505	0.96
	^{13}C	117.0	222	205	−76	298	0.89
DTU	^{13}C-4	188.9	231	189	147	−62	0.89
	^{13}C-5	137.8	236	156	22	−174	0.69
$[(TU)Cd(SeCN)_2]$	^{13}C-2	187.3	338	173	51	−205	0.81
	^{13}C-5	140.9	241	148	34	−161	0.87
	^{13}C	119.6	213	121	24	−143	0.96
	^{113}Cd	311.1	393	299	241	−104	0.70
$[(DTU)Cd(SeCN)_2]$	^{13}C-4	191.6	290	227	57	−201	0.47
	^{13}C-5	143.1	277	113	29	−166	0.55
	^{13}C	119.9	212	124	23	−146	0.91
$[(DTU)Hg(SeCN)_2]$	^{13}C-4	187.3	268	235	59	−192	0.26
	^{13}C-5	139.3	258	164	−4	−215	0.66
	^{13}C	119.2	188	125	45	−111	0.87

Notes: [a] isotropic shielding, $\sigma_i = (\sigma_{11} + \sigma_{22} + \sigma_{33})/3$; $\Delta\sigma = \sigma_{33} - 0.5(\sigma_{11} + \sigma_{22})$; $\eta = 3(\sigma_{22} - \sigma_{11})/2\Delta\sigma$.

Table 5
Solid-state ^{15}N Isotropic Chemical Shifts (δ_{iso}) and principle shielding tensors (δ_{xx})[a] of complexes Hg(II)-selenocyanate complex

Complex	Nucleus	δ_{iso}	δ_{11}	δ_{22}	δ_{33}	$\Delta\sigma$	η
DTU	^{15}N	−180.11	−81.11	−170.08	−289.14	163.54	0.82
	^{15}N	−210.16	113.15	−195.12	−322.22	168.09	0.73
[(DTU)Hg(SeCN)$_2$]	^{15}N	−144.98	−45.50	−132.85	−256.59	167.41	0.78
	^{15}N	−156.73	−27.66	−169.72	−272.80	174.11	0.73

Notes: [a] isotropic shielding, $\sigma_i = (\sigma_{11} + \sigma_{22} + \sigma_{33})/3$; $\Delta\sigma = \sigma_{33} - 0.5(\sigma_{11} + \sigma_{22})$; $\eta = 3(\sigma_{22} - \sigma_{11})/2\Delta\sigma$.

Table 6
Anti-bacterial activities of Cd(II) and Hg(II) complexes

Microorganisms	Zone of inhibition					
	Cd(SeCN)$_2$	Hg(SeCN)$_2$	TU	DTU	(DTU)Cd(SeCN)$_2$	(DTU)Hg(SeCN)$_2$
K. pneumoniae	–	22	10	20	R	R
P. aeruginosa	20	10	NR	10	NR	NR
S. aureus	20	22	NR	15	NR	NR
S. typhi	28	10	NR	20	NR	NR

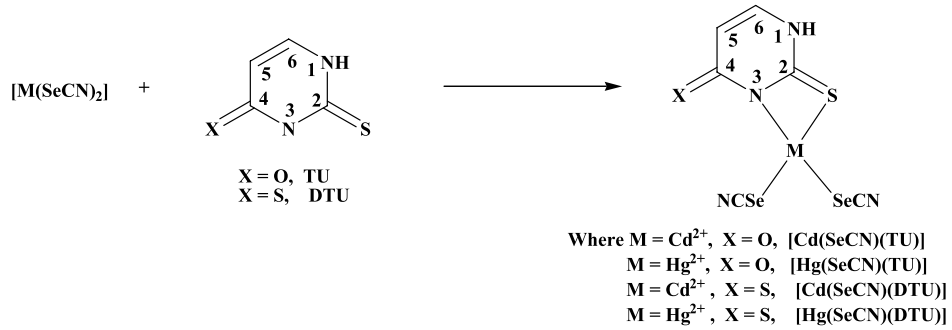
Note: NR – not reactive.

strength of 4.5, whose chemical shift was taken to be 0 ppm [14]. The same $Cd(NO_3)_2 \cdot 4H_2O$ sample was used to set up the Hartmann–Hahn condition for the CPMAS experiments. ^{13}C chemical shifts were referenced to TMS by setting the high frequency isotropic peak of solid adamantane to 38.48 ppm. The center peaks were differentiated from the spinning side-bands by recording the spectra at two different spinning speeds. The calculated ^{13}C and ^{113}Cd shielding tensors are shown in Table 4.

The ^{15}N NMR spectra were recorded using $^{15}NH_4NO_3$ as external reference [7]. The spectral conditions for ^{15}N were: 32K data points, 0.721 s acquisition time, 2.50 s delay time, 60° pulse angle and ∼5000 scans. The chemical shift of nitrogen was initially referenced with respect to liquid NH_3, by setting the ^{15}N peak in enriched solid $^{15}NH_4Cl$ to 40.73 ppm [10], and then converted to the standard nitromethane by a shift of −380.0 ppm [7] for ammonia. The selected data are shown in Table 5. The ^{113}Cd and ^{15}N spectra containing spinning side-band manifolds were analyzed using a computer program WSOLIDS developed at Dalhousie and Tubingen Universities [3].

2.3. Synthesis of Cd(II) and Hg(II) complexes

Since 2,4-dithiouracil ligand was ^{15}N enriched (∼90%) ligand, it was mixed with unlabeled 2,4-dithiouracil ligand (10:90% between label and unlabeled ligands). Solution of $CdCl_2$ in 10 ml dist water mixed with stoicheometrically one equivalent of ligand (2-thiouracil or 2,4-dithiouracil) in 10 ml solvent mixture (methanol:water = 1:1) as Scheme 1. The resulting solution was stirred for 30 min, and then two equivalents KSeCN water solution was added and the resulting mixture refluxed at 70°C under nitrogen environment with stirring for 15 min and then refluxed for another ∼1.5 h at same temperature. Produced solution filtered and kept for crystallization in the refrigerator. After 2 days the product was collected and dried under vacuum. Same procedure was applied for mercury complexes using $HgCl_2$ instead of $CdCl_2$.



Scheme 1. Synthetic route.

2.4. Anti-bacterial assay

Standard type cultures of bacteria were obtained from Microbial Type Culture Collection (MTCC) Chandigrah, India. The agar well diffusion technique [17] was used to screen the anti-bacterial activity. *In vitro* anti-bacterial activities were screened by using nutrient agar plates obtained from Himedia (Mumbai). The plates were prepared by pouring 20 ml of molten media into a sterile Petri dish and allowed to solidify for 5 min. A sterile cork borer of diameter 6.0 mm was used to make wells in the agar plates. Inoculums were swabbed uniformly on the surface of agar plates. 0.1 mg/well were loaded on 6.00 mm diameter well. Dosage of each chemical started from 100 μg/ml and continued until MIC (minimum inhibitory concentration) was reached. A maximum dose of 1000 μg/ml was used for stopping criterion. The plates were allowed to stand for 1 h for diffusion then incubated at 37°C for 24 h. At the end of incubation, inhibition zones were measured.

3. Results and discussion

3.1. IR studies

Infrared spectroscopic vibration bands for the free ligands and their corresponding cadmium(II) and mercury(II) complexes are shown in (Table 2) and Fig. 1. The ν(N–H) stretching vibrational band for the [Cd(SeCN)$_2$(TU)] complexes show the shift towards higher wave number (3447 cm^{-1}) than the corresponding free thiouracil ligand (3136 cm^{-1}) indicating the formation of complex with thiouracil. Another important observation made from IR data of the [Cd(SeCN)$_2$(TU)] and [Hg(SeCN)$_2$(TU)] is that the vibrational frequency of –NH is higher by 2 cm^{-1} than the later one indicating the stronger donation of nitrogen lone pair of electron to the Cd(II). The (–C=S and –C=O) vibration, which occurs at 1711, 1689 and 1570 cm^{-1} for the free ligands, shifts towards lower frequency (1699, 1600, 1561 for [Hg(SeCN)$_2$(TU)]). But in case of corresponding [Cd(SeCN)$_2$(TU)] complex, the IR frequency appears to be higher at 1706, 1665, 1564 cm^{-1} than that of the [Hg(SeCN)$_2$(TU)] which indicate the stronger coordination of the sulphur atom to the Cd(II). The similar observation has been made to the 2,4-dithiouracil with both the metals Cd(II) and Hg(II) upon complexation. Other important vibrational bands in IR spectra around 2127 and 2107 cm^{-1}, corresponding to CN$^-$ stretching, are observed for the Hg(SeCN)$_2$ and Cd(SeCN)$_2$ complexes, respectively, while upon complexation with 2-thiouracil and Hg(II) IR frequency shifts towards lower frequency region. The similar trends is observed with the Hg(SeCN)$_2$ and Cd(SeCN)$_2$ compounds upon complexation with 2,4-dithiouracil.

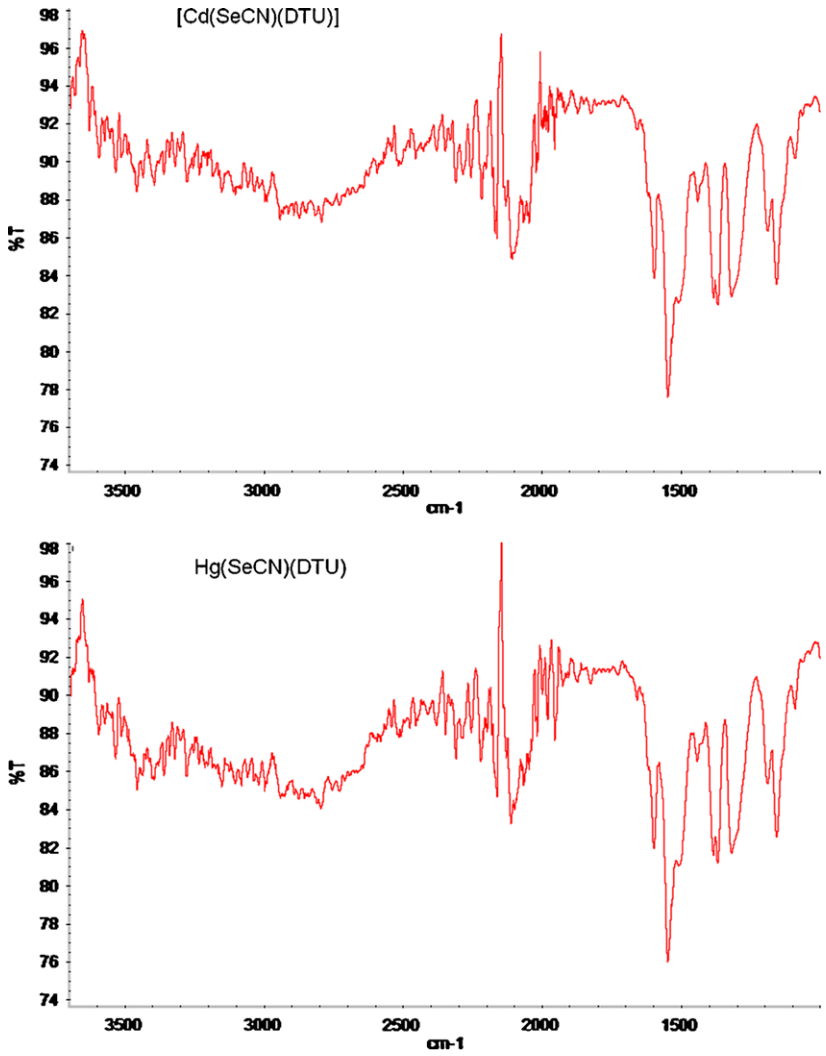


Fig. 1. IR spectra of Cd(II) and Hg(II) complexes. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0503.)

3.2. Solution NMR studies

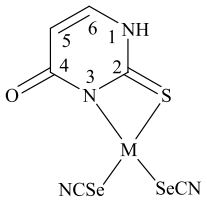
Table 3 shows the various ^{13}C NMR chemical shift of the thiouracils and corresponding Cd(II) and Hg(II) complexes. The downfield chemical shifts were observed for the prepared complexes with respect to the thiouracils. This is may be due to the σ-donation of nitrogen lone pair to the metal center that causes the deshielding of the carbons next to the bonding –C=S and –NH functional groups. But in case of the Cd(II) complexes with both ligands, 2-thiouracil as well as 2,4-dithiouracil, a downfield chemical shift was observed than that of the analogous Hg(II) complexes. This can be attributed to higher Lewis acidic nature of the Cd(II) which encourages more σ-donation than that of the Hg(II) complexes [13]. For example, as in the case of $[Cd(SeCN)_2(TU)_2]$ and $[Cd(SeCN)_2(DTU)]$ the C-2 chemical shift appears at 165.09 and 175.04 ppm, respectively, while in corresponding mercury complexes, the shift appears at 161.62 and 168.92 ppm. It is also worth to mention that in the ^{13}C of the CN^- in $[Cd(SeCN)_2]$ and

$[Hg(SeCN)_2]$ a sharp singlet was observed at 116.92 and 105.83 ppm, respectively. But upon complexation of $[Cd(SeCN)_2]$ with thiouracil, a downfield chemical shift was observed by about 3 ppm while the complexation with 2,4-dithiouracil shift appeared at 117.30 ppm.

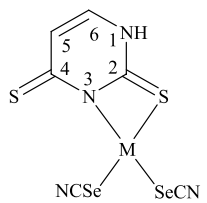
3.3. Solid-state NMR studies

The ^{13}C, and ^{113}Cd CPMAS NMR spectral data, respectively, for complexes $[Cd(SeCN)_2(TU)]$, $[Cd(SeCN)_2(DTU)]$ and $[Hg(SeCN)_2(TU)]$ and $[Hg(SeCN)_2(DTU)]$ are shown in Table 4. The solid-state ^{13}C and ^{15}N NMR spectra are shown in Figs 2–4 and the peaks are denoted by asterisk. The calculated chemical shift tensors are also compiled in Table 4, along with the span, Ω, which describes the breadth of the chemical shift tensor and skew κ describing the shape of the powder pattern. The complex, $[Cd(SeCN)_2(TU)]$, ^{113}Cd isotropic shift in the complexes moves to downfield region by about 100 ppm upon complexation with 2-thiouracil, compared to complex $Cd(SeCN)_2$. It is also observed that the thiouracil and 2,4-dithiouracil ^{13}C isotropic shift in the Cd(II) complexes moves towards downfield region upon complexation, compared to free ligand. The bonding of the thiouracil to cadmium is shown to be stronger than that of mercury. This is supported by the solid-state ^{13}C NMR data (Table 4) where it can be seen that >6 ppm deshielding shift for 2,4-dithiouracil–cadmium complex (137.8–143.1) for the isotropic peak of carbon while only <2 ppm deshielding shift (137.8–139.3) observed for mercury complex. In the case of 2-thiouracil the most preferred coordination sites is through nitrogen and sulfur [15,18] rather than oxygen. While in case of 2,4-dithiouracil coordination occurs through sulphur at C4 which matches well with literature data [12].

Based on the evidences presented by IR as well as by NMR following structures can be proposed:



Where M = Cd^{2+} $[Cd(SeCN)_2(TU)]$
M = Hg^{2+} $[Hg(SeCN)_2(TU)]$



Where M = Cd^{2+} $[Cd(SeCN)_2(DTU)]$
M = Hg^{2+} $[Hg(SeCN)_2(DTU)]$

3.4. Anti-bacterial activity

The complexes were evaluated for '*in vitro*' anti-bacterial activity against both gram positive as well as gram negative bacteria. Two complexes, $[Cd(SeCN)_2(DTU)]$ and $[Hg(SeCN)_2(DTU)]$ exhibited less anti-bacterial activity compared to $[Cd(SeCN)_2]$, $[Hg(SeCN)_2]$ and the ligand itself. The activities of the complexes are summarized in Table 6.

4. Conclusion

In this study, we have shown that thiouracils react with $[Cd(SeCN)_2]$ and $[Hg(SeCN)_2]$ to form complexes of the type $[M(SeCN)_2(TU)]$ and $[M(SeCN)_2(DTU)]$ (where M is Cd^{2+} and Hg^{2+}). Based on elemental analysis data, we propose that one metal center is surrounded by one thiouracil unit.

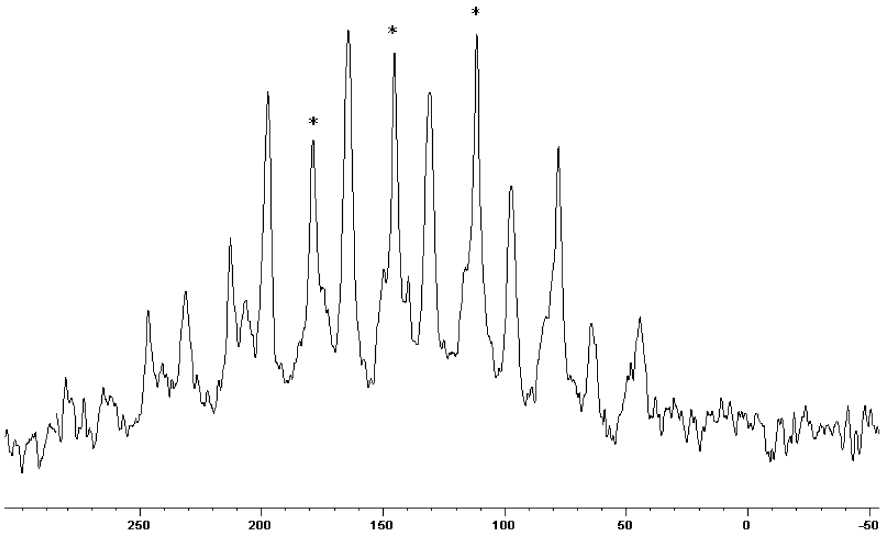


Fig. 2. ^{13}C CPMAS spectra of (a) [(TU)Cd(SeCN)$_2$]. The center peak is denoted by "*".

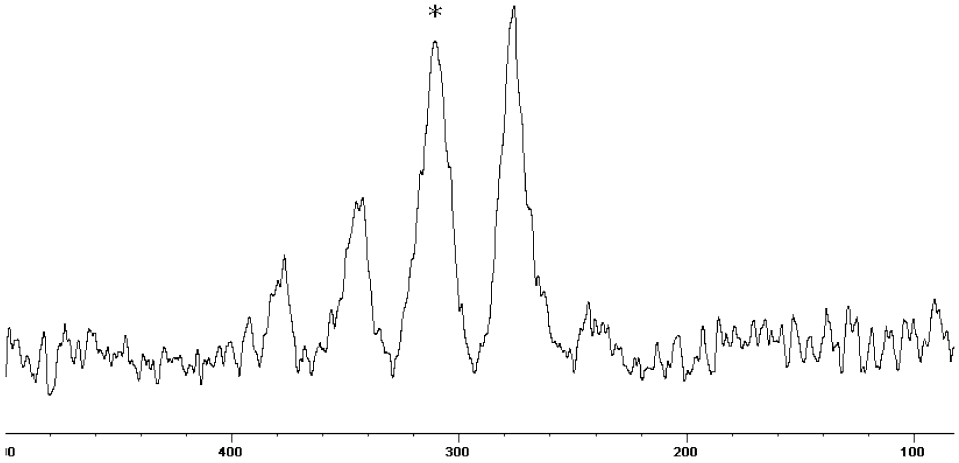


Fig. 3. ^{113}Cd SPMAS spectra of [(TU)Cd(SeCN)$_2$]. The center peak is denoted by "*".

The ^{15}N labeling studies have shown the involvement of N atom of thiouracil moieties in coordination to the metal. It is evidenced that the bonding of the thiouracil to cadmium is shown to be stronger than that of the corresponding mercury complexes. The complexes of the type [M(SeCN)$_2$(TU)] and [M(SeCN)$_2$(DTU)] have shown zone inhibition towards different microorganisms but less than that of the Cd(SeCN)$_2$ precursor and ligand itself.

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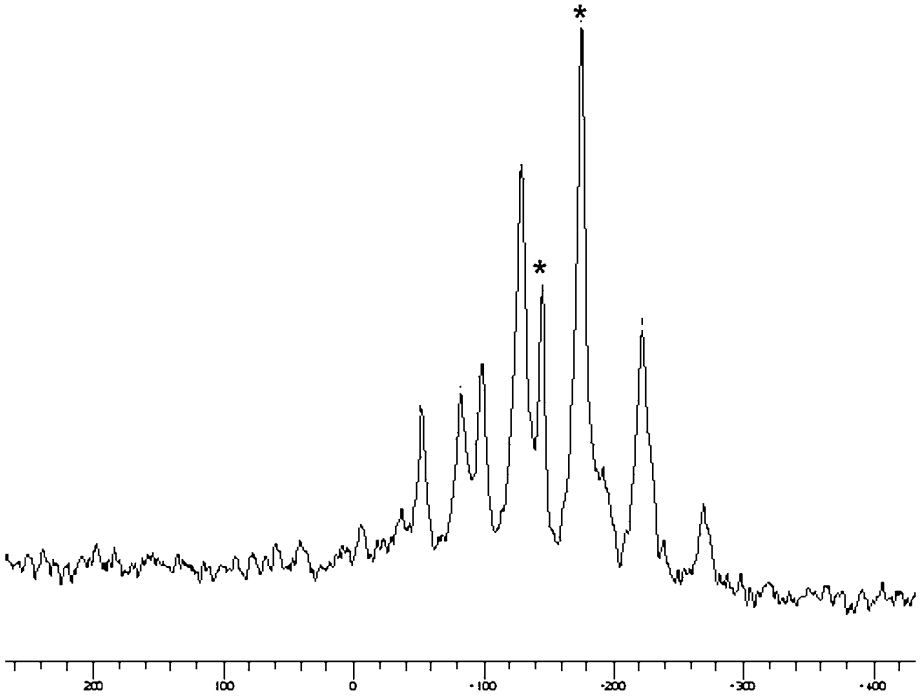

Fig. 4. ^{15}N NMR spectra of [(DTU)Hg(SeCN)$_2$]. The center peak is denoted by "*".

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