Synthesis, Characterization and Cytotoxic Properties of Pd(II), Cd(II) and Hg(II) Complexes of 2-Amino-5-Chloro-Benzophenone-4-(H/Ph)-Thiosemicarbazones

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ABSTRACT

New complexes of 2-amino-5-chloro-benzophenone-4-(R)- thiosemicarbazones [where R= H (HL^1), C_6H_5- (HL^2)] with Pd(II), Cd(II) and Hg(II) chloride salts were synthesized. The stable diamagnetic complexes with 1:1 molar ratio were characterized by analytical data, molar conductivity and magnetic measurements, IR and ^{1}H-NMR spectra. It was determined that the compounds, HL^1 and HL^2 exhibit bi- or tridentate ligand behaviour depending on the metal ion and the substituent on N^4 atom of the thiosemicarbazone. Cytotoxicity and proliferation experiments using K 562 chronic myeloid leukemia cell line and ECV 304 human umbilical vein endothelial cell line (HUVECs) imply that the Pd(II) and Hg(II) complexes with L^1 have allowable anti-leukemic effects. For K 562, the LD_{50} doses of $[Pd(L^1)Cl]\cdot C_2H_5OH$ and $[Hg(L^1)Cl]\cdot H_2O$ were determined as 3.0 and 2.4 microgram/ml, respectively.

CONTENTS

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INTRODUCTION

Metal complexes of thiosemicarbazones are a class of compounds presenting a wide range of biological applications as antiviral, antibacterial, antineoplastic and antitumor agents depending on the substituent and metal ions /1–8/. Among the metal-containing thiosemicarbazone compounds, the Pd(II) chelates have been studied regarding their antitumor potentials /9-12/. Besides, some palladium(II) complexes of thiosemicarbazones are also known as antiviral agents /13,14/. In the last years, it has been proven that some thiosemicarbazone derivatives have ribonucleotid reductase (RR) inhibitory effect. RR is a critical enzyme of RNA conversion to DNA /15/, and many papers include the investigation to determine the details of DNA binding for the metal complexes of thiosemicarbazones /16-19/.

N^4-substituted benzophenone thiosemicarbazones are effective on *E. coli*, 2-hydroxy-5-substituted derivatives and also their Cu(II) and Co(II) complexes show a bacteriostatic activity towards *Bacillus megatarium* /20/, and exclusively, 2-amino-5-substituted benzophenone thiosemicarbazones indicate an anticonvulsant activity /21/.

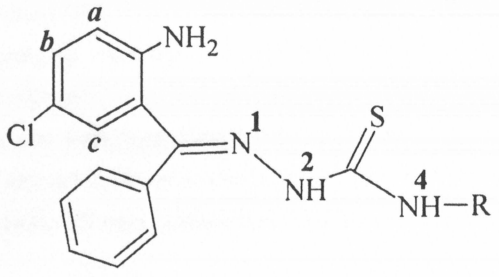


Fig. 1: 2-Amino-5-chloro-benzophenone-4-R-thiosemicarbazones, R= H (HL^1); C_6H_5- (HL^2)

In the scope of our investigations on structural and cytotoxic properties of thiosemicarbazone derivatives /22/, we present the synthesis and cytotoxic properties of 2-amino-5-chloro-benzophenone-4-(H/Ph)-thiosemicarbazones (HL^1 and HL^2) (Fig. 1), and their Pd(II), Cd(II) and Hg(II) complexes (**I-V**). The compounds were characterized by elemental analysis, magnetic susceptibility and molar conductivity measurements, IR and ^{1}H-NMR spectra. Their cytotoxic effects were tested for K 562 chronic myeloid leukemia and ECV 304 human umbilical vein endothelial cell lines.

EXPERIMENTAL

Chemicals and Apparatus

The chemicals used were reagent grade. The elemental analyses of samples were carried out by a Carlo-Erba 1106 and Varian Spectra–220/FS Atomic Absorption spectrometer. The chloride content was determined by a Jenway 3040 ion analyser. IR spectra were recorded (KBr disks) on a Mattson 1000 FT-IR spectrometer. The ^{1}H-NMR spectra were obtained from Bruker AC-200 MHz FT-NMR spectrometer (TUBITAK, Turkey) and chemical shifts were expressed in DMSO-d_6, relative to TMS. Magnetic measurements were carried out at room temperature by the Gouy technique with an MK I model device obtained from Sherwood Scientific. The molar conductances of the compounds were measured in DMF on a WPA CMD750 conductivity meter at 25±1°C.

Synthesis of the compounds

Synthesis of 2-amino-5-chloro-benzophenone-4-(R)-thiosemicarba-zones [where R= H (HL^1), C_6H_5-(HL^2)] was reported in our previous paper /22/.

[Pd(L^1)Cl].C_2H_5OH (**I**)**:** Lithium tetrachloropalladate(II) (1 mmol), prepared *in situ* from palladium chloride (1.1 mmol) and lithium chloride (2.2 mmol) in ethanol was added dropwise to the solution of 0.305 g (1 mmol) HL^1 in 5 mL ethanol /23/. The reaction mixture was stirred for 5 h at 50°C in a water bath. The resulting yellow precipitate was filtered, washed with 3 mL cold ethanol and 3 mL diethylether and dried *in vacuo* over $CaCl_2$ (yield 0.457g, 92.9 %).

The procedure mentioned above was used with small modifications in solvent volume and reaction time to prepare the complex of HL^2 with $Li_2[PdCl_4]$ (**II**).

[Cd(L^1)(H_2O)Cl] (**III**): 0.305 g (1 mmol) HL^1 was dissolved in 5 mL of ethanol by heating. This solution was treated dropwise with 10 mL of an ethanolic solution of 0.200 g (1 mmol) $CdCl_2.H_2O$. The reaction mixture was stirred for 5 h at 50°C in a water bath. The resulting white precipitate was filtered, washed with 5 mL cold ethanol and 5 mL diethylether and dried *in vacuo* over $CaCl_2$ (yield 0.378 g, 80 %).

[Cd(HL^1)Cl_2] (**IV**) and [Hg(L^1)Cl].(H_2O) (**V**) were prepared in a similar manner/24/. The analytical data of all compounds are given in Table 1.

Table 1

Analytical data and some physical properties of the compounds

Comp	Yield (%)	Colour	M.p. (°C)(*)	Λ(**)	Elemental Analysis (%) found (calculated)				
					C	H	N	Cl(***)	Metal
I	92.9	yellow	272.5	59.4	39.24 (39.08)	3.77 (3.69)	11.22 (11.39)	14.28 (14.42)	21.55 (21.64)
II	41.3	orange	264.5	17.6	45.92 (46.04)	3.25 (3.09)	10.77 (10.74)	13.78 (13.59)	20.34 (20.40)

Table 1 (continued)

III	80.0	colourless	211.2	26.1	35.92 (35.80)	3.19 (3.00)	12.06 (11.93)	15.09 (15.10)	23.85 (23.93)
IV	55.6	colourless	226.4	27.5	42.60 (42.58)	3.22 (3.04)	9.89 (9.93)	18.95 (18.85)	19.87 (19.92)
V	85.2	colourless	212.1	21.4	30.05 (30.14)	2.49 (2.53)	9.95 (10.04)	12.98 (12.71)	35.86 (35.96)

(*) decomp. (**) in DMF (Ω^{-1} cm^2 mol^{-1}). (***) inorganic chloride

Cell Cultures and Cytotoxicity Assay

The cell cultures, solutions of the thiosemicarbazone compounds and cytotoxic assays were performed as described elsewhere /25/. Cytotoxic effects of the compounds were evaluated by MTT (Sigma M-5655) test for K 562 chronic myeloid leukemia cell line and ECV 304 human umbilical vein endothelial cell line. Colorimetric analysis was performed by ELISA multiwell spectrophotometer (Diagnostics Pasteur LP 400). Data were calculated according to the % cytotoxicity formula and expressed as mean±S.D. Statistical analysis was performed using the Statistical Package for Social Statistics (SPSS). Student's t test was used to compare K 562 cells to ECV 304 cells, with $p< 0.05$ considered a significant difference. Lethal concentration 50 values (LC_{50} is the concentration that kills 50 % of the cells) were derived by interpolation from log-linear plot of concentration-toxicity (Table 3).

Table 2

^{1}H-NMR data of metal complexes

Compound	o-NH_2	N^2H	N^4H_2 (or N^4H)	Aromatic protons		
				a	b	c
I (*)	6.51 d	8.18 s	10.64 s (1H)	7.41 d	7.26 d	7.23 s
II	6.81 d	-	9.71 s (1H)	7.53 d-d	6.94 d	6.91 s
III	5.32 s	8.64 s	9.82 s (1H)	7.30 d	6.91 d	6.87 s
IV	5.47 s	9.05 s	10.28 s /10.36 s cis/trans (1H)	7.31 d	6.98 d	6.94 d
V	6.83 s	9.02 s	9.27 s (1H)	7.26 d-d	6.91 d	6.87 s

(*) Alcohol protons are at 1,05 ppm (3H, *t*) and 3.44 ppm (2H, *q*)

Table 3

Cytotoxicity Indexes (CI) and Lethal Concentrations (LC 50)

Cell Line	Compounds	5 μgr/ml	1 μgr/ml	0.1 μgr/ml	0.01 μgr/ml	LC_{50}
K 562	L^1	27,29 ± 7,25*	13,41 ± 5,69*	10,01 ± 3,59	12,10 ± 5,14	>5
	L^2	16,94 ± 7,71*	15,73 ± 4,74*	11,64 ± 4,56*	14,83 ± 6,30	>5
	(I)(*)	6,73 ± 4,42*	-5,88 ± 6,94*	3,36 ± 8,40	6,13 ± 4,41	>5
	(II)	20,78 ± 4,73	-1,53 ± 7,13	2,57 ± 3,39	1,91 ± 7,02	>5
	(III)	93,10 ± 2,40*	27,04 ± 4,01*	2,5 ± 5,26	5,5 ± 5,69	2.4
	(IV)	-22,24 ± 3,81*	-21,66 ± 9,3*	-29,6 ± 14,4*	-20,95 ± 7,48*	>5
	(V)	81,81 ± 2,73*	18,78 ± 5,52*	-1,19 ± 9,81	0,92 ± 5,85	3
	DMSO(**)	-2,18 ± 4.12	-4,30 ± 5.44	3.5 ± 2.62	2.07 ± 4.92	-
ECV 304	L^1	14,24 ± 4,61	6,46 ± 3,77	7,91 ± 2,27	7,23 ± 3,89	>5
	L^2	43,65 ± 6,41	2,84 ± 4,07	3,85 ± 4,40	5,88 ± 2,00	>5
	(I)	20,39 ± 8,47	11,73 ± 6,82	10,10 ± 3,65	11,16 ± 4,97	>5
	(II)	28,23 ± 7,84	2,55 ± 2,96	0,67 ± 4,18	3,20 ± 3,43	>5
	(III)	38,98 ± 9,53	4,08 ± 4,01	5,48 ± 1,64	9,10 ± 3,44	>5
	(IV)	12,69 ± 4,10	4,30 ± 2,12	4,53 ± 0,96	-0,92 ± 2,30	>5
	(V)	49,45 ± 15,83	7,45 ± 0,50	1,71 ± 1,94	-1,66 ± 8,03	~5
	DMSO(**)	-4,94 ± 4,32	-4.57 ± 7,94	-1.38 ± 6,36	-2,39 ± 7,90	-

(*)Mean differences is significant between K 562 and ECV 304(**) Control DMSO is equal to DMSO concentrations which are prepared of thiosemicarbazone solution

RESULTS AND DISCUSSION

Synthesis and some physical properties

The ligands, HL^1 and HL^2, are light yellow crystals, and very soluble in chlorinated hydrocarbons and polar solvents. The interaction of the Pd(II), Cd(II) and Hg(II) salts with the ligands in 1:1 molar ratio in ethanol yielded stable solid complexes, $[Pd(L^1)Cl]\cdot C_2H_5OH$ (**I**), $[Pd(L^2)Cl]$ (**II**), $[Cd(L^1)(H_2O)Cl]$(**III**), $[Cd(HL^2)Cl_2]$(**IV**), and $[Hg(L^1)Cl]\cdot H_2O$(**V**) (Table 1). The Hg(II) complexes of HL^2 could not be isolated with sufficient yield and purity. Although the purity has been improved by recrystallization from various solvent mixtures, the analytical data are not of acceptable values.

The Pd(II) complexes are yellow or orange, while the Cd(II) and Hg(II) complexes are colourless. The complexes form as a mixture of amorphous and very fine crystal material. All of the metal complexes are easily soluble in polar solvent such as DMSO and DMF, sparingly soluble in alcohols, whereas they are insoluble in chlorinated hydrocarbons. The molar conductivities of the metal complexes in 10^{-3}M DMF solution are in the range 21-29 Ω^{-1} cm^2 mol^{-1}.

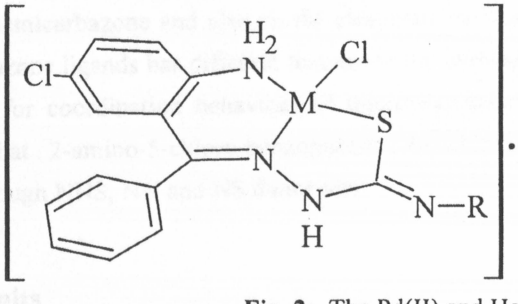
The Pd(II) and Hg(II) complexes

In the infrared spectra of the thiosemicarbazone ligands, the bands in the region 3395-3192 cm^{-1} were clearly monitored and attributed to symmetric and asymmetric stretching vibrations of the *o*-NH_2 group. These bands are weakened and shifted to lower frequencies by *ca.* 5-15 cm^{-1} in the infrared spectra of the complexes, **I, II** and **V. A** fundamental change was observed in the 1590-1510 cm^{-1} region which includes $\delta(N^2H)$ and combination bands of thioamide. Besides, it is noticable that the $\nu(C{=}N^1)$ band shifted from 1624 cm^{-1} to 1620 cm^{-1} and altered to a broadened band. These medium broad bands can be accepted as an evidence due to the $C{=}N^1$ and deprotonized sulphur atom coordinated to the Pd(II) or Hg(II) ions.

In the ^{1}H-NMR spectra of these complexes, the singlet signal of the *o*-NH_2 protons shifted to higher frequencies by *ca.* 1.20-1.50 ppm compared to the free ligands. The integral value of N^4 proton (equal to 1 H) in the spectra of L^1 complexes indicates a deprotonisation on the sulphur atom. In these circumstances, a new double bond is constituted between C^3 and N^4, and therefore their transposition of double bond causes considerable changes of chemical shifts of N^2H and N^4H protons (Table 2).

As an interesting result, the ^{1}H NMR spectra of the palladium complex **I** includes a triplet at 1.05 (3H) and a quartet at 3.44 (2H) that are evidence for coordinated alcohol molecules (uncoordinated ethanol gives resonances at 1.22 and 3.70 ppm, respectively) /24/. With a detailed analysis of proton resonance spectra of complex **I,** it was determined that the resonances of ***a***, ***b*** and **c** protons shifted to higher frequencies compared to the free ligand. The bonding between Pd(II) ion and $C{=}N^1$ and *o*-NH_2 groups should be powerful, so that the conjugated electronic system of the ligand, and therefore the electron delocalization in the aromatic ring have been noticeably affected. However, by formation of complex **II**, the changes in the chemical shifts of these protons, ***a***, ***b*** and ***c***, are in lower values in comparison with the complex **I**. In the palladium complexes, it can be said that the N^1 and N^4 atoms of HL^1 is bonded stronger than the nitrogen atoms of HL^2.

The IR and ^{1}H-NMR data indicated tridentate behaviours of the ligands in the Pd(II) and Hg(II) complexes, as shown in Figure 2.



M	R	S	
Pd	H	C_2H_5OH	**(I)**
Pd	Ph	—	**(II)**
Hg	H	H_2O	**(V)**

Fig. 2: The Pd(II) and Hg(II) complexes

The Cd(II) complexes

In the infrared spectra of the cadmium complex **III**, no fundamental changes in the stretching and deformation bands of the *o*-NH_2 group were observed. However, in the 1590-1510 cm^{-1} region which

includes $\nu(C=N^1)$ and also combination bands of thioamide, a new pattern was observed because of tautomerism in the thioamide group ($-N^2H-C=S \Leftrightarrow -N^2=C-SH$) /26/. The spectra of $[Cd(HL^2)Cl_2]$ showed that the stretching vibrations of the *o*-NH_2 weakened and shifted to lower frequencies compared to the free ligand. But, in these spectra, a similar pattern was observed in the 1580-1510 cm^{-1} region in spite of some minor differences. In the spectra of $[Cd(L^1)(H_2O)Cl]$, the chemical shifts of the protons on *o*-NH_2 group and aromatic ring changed only 0.05 ppm by complexation. But, the protons of N^2 and N^4 shifted to high field *ca.* 0.3 and 1.2 ppm, respectively, and each of the measured integral values are equivalent to one proton. In the spectra of complex **IV**, the chemical shifts of the N^2 and N^4 protons did not change compared to free ligand, and also its isomer peaks of the N^4H could be recorded identically. Besides, the *o*-NH_2 and aromatic *a* protons resonances showed considerable shifts to lower frequencies due to coordination of the *o*-NH_2 group.

Considering the spectral data it can be seen that the studied cadmium complexes have different structural characteristics. The ^{1}H-NMR data also confirms the two different formations of the Cd(II) complexes, **III** and **IV**, in Fig. 3. The spectroscopic evidence indicates the bidentate behaviour of the ligands towards Cd(II) ion. HL^1 coordinates through N^1 and deprotonized sulphur atoms while HL^2 coordinates through N^1 and *o*-NH_2 nitrogen.

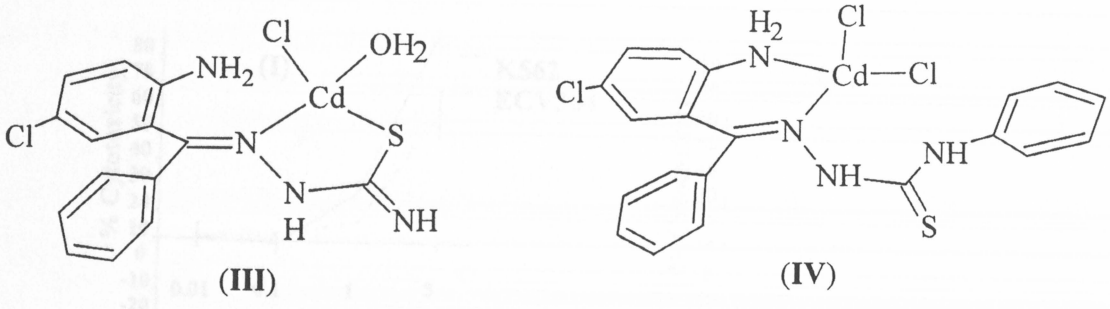


Fig. 3: The proposed structures for the Cd(II) complexes

As known, coordination behaviour of thiosemicarbazone derivatives depends on the N^1, N^4 and *S*-substituents of thiosemicarbazone and also on the electronic configuration of the metal ion. Therefore, each of the thiosemicarbazone ligands has different features in the chelation sense, and it is impracticable to obtain generalized results for coordination behaviour of thiosemicarbazone ligands. By the investigation, it has become evident that 2-amino-5-chloro-benzophenone-4-(H/C_6H_5)-thiosemicarbazones have a versatile chelating ability through NNS, NN and NS donor sets.

Cytotoxicity Results

The cytotoxicity assays indicated that all compounds are more cyototoxic for K 562 than for ECV 304. The ligands have low cytotoxic effect on both cell lines. In 5 µg/ml concentration, HL^2 has significantly higher cytotoxicity for ECV 304 than K 562 (Table 3 and Fig. 4). For K 562 and ECV 304 cells, the lethal concentration 50 % (LC_{50}) of the ligands and complexes are higher than 5 microgram/ml, except the Hg(II)

and Pd(II) complexes of the HL^1 ligand. The LC_{50} values of the Hg(II) and Pd(II) complexes for K 562 cell line are 2,4 and 3,0 microgram/ml, respectively. These results suggested that the Pd(II) and Hg(II) complexes have considerable anti-leukemic effects on leukemic cell line, these complexes show also significantly lower cytotoxic effects for non-cancerous endothelial cell line at the same doses (Fig 4).

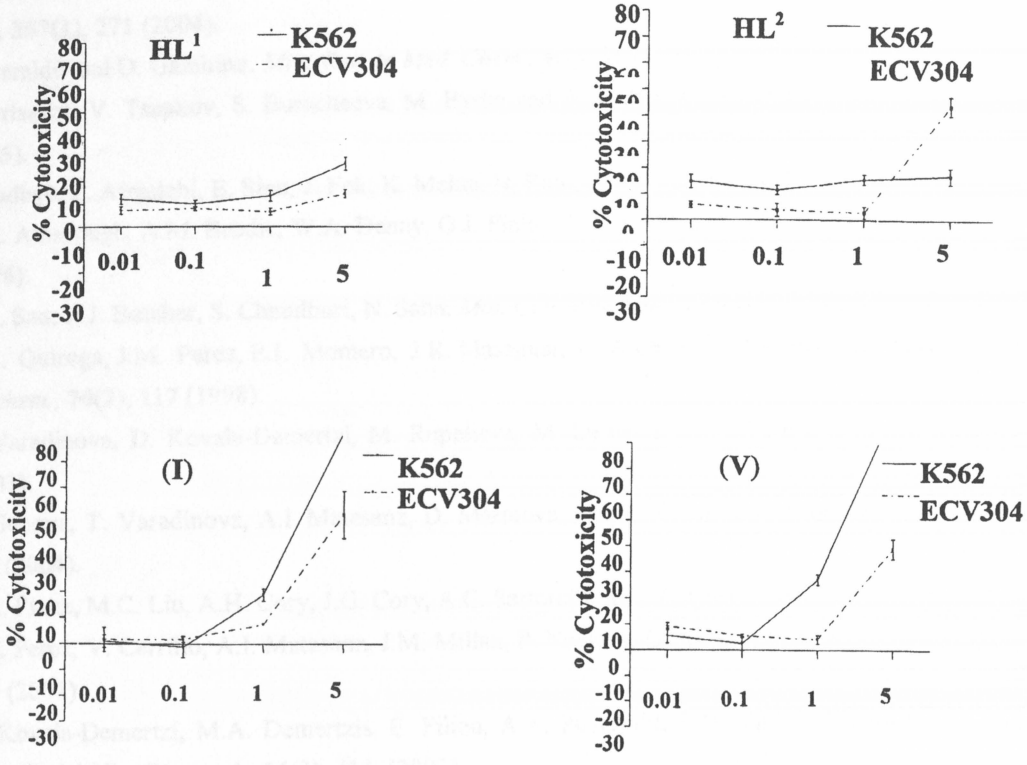


Fig. 4: Cytotoxic effects of HL^1, HL^2, complex **I** and **V**

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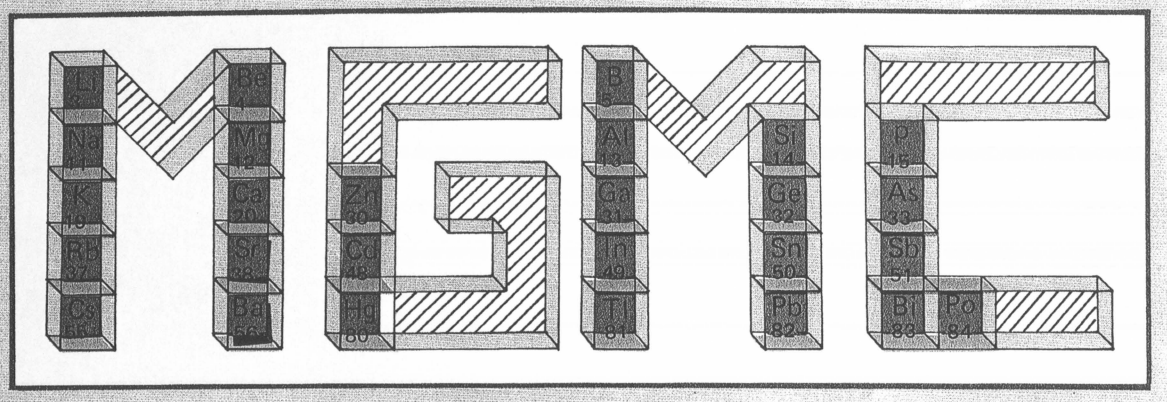

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