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SYNTHESIS OF BENZOIMIDAZOLE SELENATO COMPLEXES AND DETERMINATION OF ITS ANTITUMOR ACTIVITY

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ABSTRACT

Different selenium complexes were synthesized using benzimidazole selenato ligand of Zn (II), Hg (II), Cd (II), Cu (II) and Ni (II). The derivatives M [Se (BIA)]$_2$ (where M = Zn, Ni, Cu, Cd, Hg and BIA- benzimidazole) were prepared by the reaction of MCl_2 with lithium arene selenato ligand of $BIASe^+Li^-$. The compounds synthesized were identified and characterized by various methods like melting point, thin layer chromatography, nuclear magnetic resonance, mass spectroscopy, atomic absorption spectroscopy, and screening for anti tumor activity was carried out of all the derivatives using Ehlirch asicites carcinoma (EAC) cells induced in albino mice. Out of the complex synthesized, selenato metallic complexes Zn[Se(BIA)]$_2$ and Ni[Se(BIA)]$_2$ were found to be potent antitumor, since these compounds have shown increase in RBC, decrease in WBC, increase in life span , decrease in ascities fluid volume , increase in lymphocytes significantly.

Key words: Selenium complexes, Benzimidazole, Ehlirch asicites carcinoma (EAC)

INTRODUCTION

In last few years, there has been considerable interest in the synthesis and characterization of metal complexes of ligands derived from heterocyclic molecule because of their biological significance[1]. The metal complexes especially of Pd (II) and Pt (IV) with various heterocyclic compounds as ligands have also been studied by researchers due to their antitumor, antiviral, antibacterial and antifungal activities[2]. Various metal complexes of heterocyclic compounds and their Ni (II), Pd (II), Pt (I) and Cu (II) complexes have also been synthesized. Heterocyclic ring system like benzimidazole and few metals like Zn (II,)

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Pt (II), Cu (II) and Ni (II) have also known to possess antitumor activity. As now-a-days, organo selenium complexes especially of group 6 metals have attracted current attention for antitumor activity[3]. There exists Se-N intramolecular coordination in these complexes, which is responsible for its stability. This coordination has also shown the possibility that this interaction may play an important role in the catalytic cycle of glutathione peroxidase, a selenium containing anti-oxidation enzyme[4]. Selenium is also known to be anti-mutagenic and anti-carcinogenic agent[5]. So, attempts have been made to synthesize benzimidazole selenato metallic complexes and screen them for antitumor activity using Ehlirch asicites carcinoma (EAC) cells induced in albino mice.

EXPERIMENTAL

Material and method

All the reactions were carried out under nitrogen atmosphere. The nitrogen gas was obtained from nitrogen cylinder using pyragallol solution (for the removal of moisture and oxygen), KOH pellets and concentrated H_2SO_4 (for the removal of water)[6,7]. This nitrogen free of moisture and oxygen was then filled into a balloon fitted with a take off. Solvents were purified by standard procedure and were freshly distilled prior to use. Melting points were recorded by capillary tube method[8,9]. IR spectra were recorded on a Shimadzu-IR spectrophotometer using KBr pellets. NMR spectra were carried on Brucker 200 spectrospin using TMS as internal standard. Mass spectra were recorded using electron impact (EI) method[10–12]. Atomic absorption spectroscopy was carried out at Indian Bureau of Mines, Bangalore. The structures of all the compounds were consistent with their analytical and spectral data. Antitumor screening was carried out using Ehlirch asicites carcinoma (EAC) cells induced albino mice brought from Kasturba Medical College, Manipal and were maintained *in vivo* in mice by i.p every 10 day. The permission of conducting this experiment was obtained from Institutional Animal Ethics Committee.

Synthesis of $M[Se(BIA)]_2$ (IV) Where M = Zn, Ni, Cu, Cd and Hg

To a solution of benzimidazole (1 g, 10 mmol) in dry tetrahydrofuran 20 mL, 1.6 M solution of n-butyl lithium in hexane (6.8 mL, 11 mmol) was added under nitrogen atmosphere at -78° C. After 1hr of stirring, a white precipitate of lithated product was obtained[13]. The supernatant solvent was removed with the help of a syringe. The white precipitate was dissolved in dry methanol. The solution was cooled to 0°C and selenium powder (0.8 g, 10 mmol) was added. After 2 hrs of stirring at this temperature, anhydrous MCl_2 like Zn (II), Cd (II), Hg (II), Ni (II) or Cu (II) (5m mol) was added and stirring was continued for an additional 1 hr at 0°C and for 18 hrs at room temperature. The resulting

solution was filtered and solvent evaporated. The crude solid was recrystallized using organic solvent 1,4-dioxane to obtain the crystallized complexes $M[Se(BIA)]_2$. The reaction scheme is shown in Fig. 1.

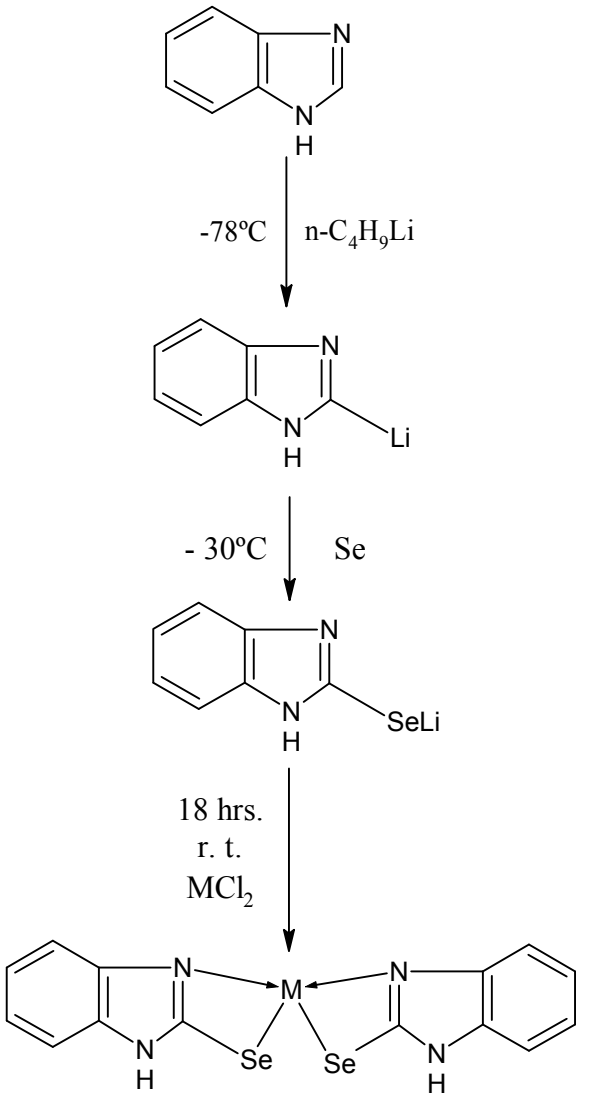

Fig. 1: Reaction scheme

Zn complex (IVa): IR (cm^{-1}): 3057.58 (Ar. C – H st.), 1455.99 (C=N st.), 1363.43 C– N st., 3422.06. (N – H st.)

^{1}H NMR (δ): 7.34-7.37 (-CH, t, aromatic), 7.44-7.47 (-CH, t, aromatic), 7.78-7.80 (-CH, d, aromatic) and 7.94-7.96 (-CH, d, aromatic).

Ni complex (IVb): IR (cm^{-1}): 3058.88 (Ar. C – H st.), 1452.29 (C=N st.), 1368.41 (C – N st.), 3421.06 (N – H) st.).

^{1}H NMR (δ): 7.33-7.37 (-CH, t, aromatic), 7.43-7.49 (-CH, t, aromatic), 7.78-7.82 (-CH, d, aromatic), 7.96-7.98 (-CH, d, aromatic).

Cu complex (IVc): IR (cm^{-1}): 3060.58 (Ar. C – H st.), 1449.99 (C=N st.), 1363.55 (C – N st.), 3428.06. (N – H) st.)

^{1}H NMR (δ): 7.34-7.39 (-CH, t, aromatic), 7.43-7.49 (-CH, t, aromatic), 7.77-7.81 (-CH, d, aromatic) and 7.93-7.98 (-CH, d, aromatic).

Cd complex (IVd): IR (cm^{-1}): 3050.51 (Ar. C – H st.), 1456.44 (C=N) st.), 1358.03 (C – N) st.), 3432.60 (N – H) st.).

^{1}H NMR (δ): 7.33-7.39 (-CH, t, aromatic), 7.43-7.49 (-CH, t, aromatic), 7.76-7.82 (-CH, d, aromatic), 7.93-7.98 (-CH, d, aromatic).

Hg complex (IVe): IR (cm^{-1}): 3051.34 (Ar. C – H st.), 1453.79 (C=N) st.), 1366.11 (C – N) st.), 3450.24 (N – H st.).

^{1}H NMR (δ): 7.33-7.38 (-CH, t, aromatic), 7.43-7.48 (-CH, t, aromatic), 7.75-7.81 (-CH, d, aromatic), 7.93-7.99 (-CH, d, aromatic).

Antitumor activity

8-10 Week old albino mice of either sex with an average body weight of 20-25 g were maintained in identical condition and fed with standard food pellets and water. EAC cells were maintained *in vivo* in the mice by injecting every 10 days. EAC cells of 10 days old mice were used for the studies. Challenge dose of EAC cells was given i.p. at the rate of 1 x 10^6 cells/20 g bodyweight of the animal[14]. The animals were divided into 13 groups each containing 8 mice. Animals of group I-III were kept as saline control (5 mL/kg bodyweight i.p), Ehrlich asities carcinoma (EAC) control (1 x 10^6 cells/20 g bodyweight per mice i.p), Ehrlich asities carcinoma (EAC) control (1 x 10^6 cells/20 g bodyweight per mice i.p) + DMSO (vehicle control), respectively. 5 Derivatives were dissolved in DMSO and were administered i.p. at the dose of 5µg / 20g of body weight in the group (IV-VIII).

All the compounds were administered for 9 days starting 24 hrs after tumor implantation. Four animals from each group were scarified 24 hrs after the last dose and asities fluid volume, packed volume and hematological parameters were noted[15].

Mean survival time for remaining four mice of each group was noted for 6 weeks. Asities volumes was noted by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuge at 1000 r.p.m. for 5 mins. Viability of asities cells was checked by trypan blue (0.4% in normal saline) dye exclusive test and the counts were taken in Neubauer chamber. The effect of derivatives on tumor growth was monitored by recording the mortality daily for 6 weeks and percentage increase in life span (% ILS) was calculated[16]. An enhancement by 25% or more was considered as effective antitumor response. Hematological studies were carried out by obtaining blood from tail vein 24 hrs after the last dose. For total blood count blood was drawn into RBC or WBC pipettes in proper dilution and counted in Neubauer counting chamber. Hemoglobin concentration was determined by Sahli's hemoglobinometer method[17]. Different count of leukocytes (DLS) was done on freshly drawn blood film using Leishmans stain. The most essential parameters that confirm the potency of antitumor agent are percentage increase in life span (Table 1), decrease in asicites fluid volume, (Table 2), increase in RBC, decrease in WBC and increase in lymphocytes[18] (Table 3).

Table 1. Effect of derivatives on survival time on EAC bearing mice

Group number	Design of experiment	Mean survival time (MST) (days)	% Increase in life span (% ILS)
I	Normal saline 5 mL/kg	-	-
II	EAC only (10^6 cells/mouse)	14	-
III	EAC + vehicle	14	-
IV	Zn-BIA	27	84.93
V	Ni- BIA	24	64.38
VI	Cu-BIA	23	57.53
VII	Cd- BIA	19	30.13
VIII	Hg- BIA	17	18.28

Table 2. Effect of derivatives on Asicites fluid volume and tumor cell count in EAC induced mice

Group number	Design of experiment	Total ascites fluid volume (mL)	Packed tumor cell volume (mL)	% Viable cell in ascites
I	Normal saline 5 mL/kg	-	-	-
II	EAC only (106 cells/mouse)	7.2	3	95.72
III	EAC + vehicle	6.8	2	92.58
IV	Zn- BIA	3.8	1.12	22.01
V	Ni- BIA	4	1.35	38.21
VI	Cu- BIA	4	1.26	32.01
VII	Cd- BIA	3	1.58	43.23
VIII	Hg- BIA	5	1.27	41.9

Table 3. Effect of derivates on Haematological parameters in tumor bearing mice

Group number	Design of experiment	Hb (gm/dL)	RBC count (x 10^6)	WBC count (x 10^6)	Lymphocyte (%)	Neutrophile (%)	Monocyte (%)
I	Normal Saline 5 mL/kg	13.00	5.26	6.86	58.00	30.0	2.0
II	EAC only (106 cells/mouse)	11.00	7.33	23.23	42.00	74.0	1.0
III	EAC + vehicle	10.00	6.58	11.22	42.90	60.0	1.0
IV	Zn- BIA	11.00	7.40	19.62	48.21	53.0	1.0
V	Ni- BIA	10.21	6.21	16.86	62.68	42.0	1.2

Cont…

Group number	Design of experiment	Hb (gm/dL)	RBC count (x 10^6)	WBC count (x 10^6)	Lymphocyte (%)	Neutrophile (%)	Monocyte (%)
VI	Cu- BIA	9.00	4.72	20.52	48.20	41.0	1.2
VII	Cd- BIA	10.90	5.23	21.20	58.96	28.0	2.0
VIII	Hg- BIA	11.00	5.19	17.23	55.23	49.0	2.0

Table 4. Data of various derivatives

Comp code	Molecular formula	Mol wt.	M. P. (°C)	% Yield	R_f Value
IVa	Zn $[Se(BIA)_2]$	457.5	162	15	0.38
IVb	Ni $[Se(BIA)]_2]$	450.88	162	15	0.38
IVc	Cu $[Se(BIA)]_2]$	455.73	160	16	0.42
IVd	Cd $[Se(BIA)]_2]$	504.58	155	14	0.36
IVe	Hg $[Se(BIA)]_2]$	592.78	165	12	0.4

RESULTS AND DISCUSSION

Different selenium complexes were synthesized using benzimidazole **(I)** which was lithated at 2-position by n-butyl lithium at -78° C under nitrogen atmosphere to give lithated benzimidazole **(II)**. Then chalcogen Se was introduced to this lithated benzimidazole to get lithium benzimidazole selenato ligand BIA Se^+ Li^- **(III)**. To this solution MCl_2 like, $ZnCl_2$, $NiCl_2$, $HgCl_2$, $CdCl_2$, or $CuCl_2$ was added, to yield the final products named benzimidazole selenato metallic complexes **(IV)** as **(IV)A**, **(IV)B**, **(IV)C**, **(IV)D**, **(IV)E** derivatives. These compounds were then characterized by, melting point, R_f, (Table No 4) I.R, NMR and Atomic absorption spectroscopy. EAC Cells induced albino mice were used to screen them for anti-tumor. All the compounds were administered daily for 9 days starting 24 hrs after tumor implantation. Four animals from each group were sacrificed 24 hrs after the last dose and various parameters were noted. The assessment of the drug effect was done by counting total packed cell volume (TPCV) and survival time. The compounds **(IVa)** $Zn[Se(BIA)]_2$ was found to be potent while **(IVb)** and **(IVc)**

$Ni[Se(BIA)]_2$ and $Cu[Se(BIA)]_2$, were moderate and **(IVd)** and **(IVe)** $Cd[Se(BIA)]_2$ and $Hg[Se(BIA)]_2$ were inactive.

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