http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 5(3): 335-337, 2002

Antineoplastic Activity of 2-oxo Benzylidine (3-oxo Aniline) Cu(II) Ethylenediamine

M. Saidul Islam, M. Akhter Farooque and M.A.K. Bodruddoza
Department of Chemistry, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract: New complex of Cu^{+2} ion with Schiff base derived from the condensation of m-aminophenol with o-hydroxybenzaldehyde has been synthesized. The complex has the formulae [Cu(L)(NN)]. The complex was characterized on the basis of elemental analyses, melting points, conductance and magnetic measurements, infra-red and electronic spectra. The physico-chemical studies suggest a square planar structure for Cu^{+2} complex. Antineoplastic activity of this complex has been carried out. The antineoplastic activity of the complex was carried out on Swiss Albino male mice.

Key words: Antineoplastic activity of Schiff base complex

Introduction

Cancer is defined as a neoplastic growth that has the ability to invade the surrounding tissue and disseminated by the blood stream and lymphatics (James *et al*., 1995). The cells of the body normally remain under strict developmental control. Cells which undergo rapid abnormal and uncontrolled growth at the cost of remaining cells are called neoplastic cells. The growth resulting from the division of such cells are called neoplastic growth or tumor (Power, 1981). The tumor masses are generally composed of many millions of cells and the degree of damages to the host is dependent both on the rate of cell division and the anatomic locus of the tumors (Busch, 1974). The critical factor in inducing cancer seems to be the inappropriate activation of one or more proteins that regulate cells division, transforming cells to state of cancerous growth (Voet *et al*., 1990). The treatment of cancer (Clark *et al*., 1989, Mycek *et al*., 1997 and Gilman *et al*., 1991) as we know today is generally regarded as being of the following types-
(I) Surgical removal, ii) radiotherapy and iii) chemotherapy. Certain complexes of platinum exhibit potent antitumor activity for ehrlich ascites carcinoma and leukemias (Harder *et al*., 1970 and Welsch, 1971). Keeping these facts in view, new mixed ligand complex, 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine ([Cu(L)(NN)]) has been prepared and characterized and also tested for antineoplastic activity.

Materials and Methods

The project has carried out for 6 months duration. Swiss Albino male mice of 6-8 weeks of age (weighing 20-25 gm) were collected from International Center for Diarrheal Diseases Research, Bangladesh. Transplantable tumor (Ehrlich Ascites Carcinoma) were obtained from Indian Institute of Chemical Biology, Calcutta 700032, West Bengal, India. All steps of the work were carried out at the Laboratory, Department of Biochemistry, Rajshahi University, Bangladesh.
Ten groups of Swiss Albino mice were inoculated with 140×10^4 EAC cells/mouse (i.p.) on day 0. Treatment with complex [2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine] at the doses of 2, 8 & 16 mg/kg were started 24 hours after inoculation and continued for 10 days. Normal groups of mice (not EAC inoculated) were treated similarly. One group of mice (not EAC inoculated) was treated with DMSO. The ALKP activity was assessed on day 12 and compared with those of normal group (treated and untreated), bleomycin (as standard) and control group. Number of mice per group were 4.

***In vivo* assessment of the compound as an antineoplastic agent:** *In vivo* antineoplastic activity of the test compounds was determined by measuring the effect of the test compounds on tumor cell growth inhibition, survival time of tumor bearing mice, increase in body weight of tumor induced mice, on the enhancement of total peritoneal cells in normal mice, hematological parameters and serum alkaline phosphatase activity of tumor bearing mice.

Preparation of Schiff base: The Schiff base was prepared by the condensation of *o*-hydroxybenzaldehyde with *m*-aminophenol. *o*-Hydroxybenzaldehyde (1.7 gm, 0.014 M) in absolute ethanol (20 ml) was added to an ethanolic solution (30 ml) of *m*-aminophenol (1.5 gm, 0.014 M). The mixture was heated to reduce the volume to 25 ml, then it was cooled in an ice-bath. The black crystalline product was filtered and washed with hot ethanol. The prepared Schiff base was obtained in pure form, after the treatment of column chromatography which shows a single spot in TLC.

2-Hydroxy benzylidine-3-hydroxy aniline: Preparation of [Cu(L)(NN)]: Twenty five ml of an ethanolic solution of the metal chloride (0.005 M) [$CuCl_2.2H_2O$] was added to 30 ml of an ethanolic solution of the above prepared Schiff base (1.05 gm, 0.005 M). Then 20 ml of an ethanolic solution of [NN] (0.005 M) was added to the metal salt-Schiff base solution. The resulting mixture was boiled on a water-bath for 5 minutes and cooled. The complex separated was washed with hot ethanol and dried in *vacuo* over P_4O_{10}. The prepared complex was obtained in pure form after the treatment of column chromatography which shows a single spot in TLC in the case.

Preparation of stock solution of the test compounds [2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine and Bleomycin antibiotic: For therapeutic treatment, stock solution of Bleomycin was made in distilled water at the concentration of 0.006 mg/ml. It was used as a standard treatment. 2-oxo benzylidine-(3-oxo aniline) Cu(II) ethylenediamine was dissolved in DMSO at the concentrations of 0.4, 1.6 and 3.2 mg/ml.
The bleomycins are water-soluble glycopeptide antibiotics isolated from cultures of *Streptomyces verticillus*. These compounds differ only in their terminal amine moieties. "Bleomycin" refers to the commercial preparation, which is a mixture, the predominant component (approximately 50 percent) being bleomycin A_2. Bleomycin is unique among the commercially available antitumor antibiotics in that it produces very little bone marrow depression. It has been found to be particularly useful in the treatment of testicular carcinoma, squamous cell carcinomas, and lymphomas.

Results and Discussion

The following scheme expresses the preparation of the complex.

$$CuCl_2.2H_2O + H_2L \rightarrow [Cu(L)] + 2H_2O + 2HCl \quad \ldots\ldots(1)$$

$$[Cu(L)] + NN \rightarrow [Cu(L)(NN)] \quad \ldots\ldots(2)$$

Where H_2L = Schiff base, NN = ethylenediamine.

Elemental analyses along with other data are presented in Table 1. The molar conductivity was measured in dimethyl sulfoxide (DMSO). The conductance value revealed that the complex behave as 1:1 electrolytes (Geary, 1971). The magnetic measurement indicates that the complex is paramagnetic and shows magnetic moment of 1.89 B.M. corresponding to one unpaired electron.

Table 1: Analytical data and physical properties

Complex	Colour	Metal %	M.P.	Molar conductance (Ohm^{-1} cm^2mol^{-1})	Magnetic moment in B.M.
[Cu(L)(NN)]	Greenish	18.86 (17.99)	208-210	40	1.89

Table 2 : Effect of Test Compounds on EAC (Ehrlich Ascites Carcinoma) Cell Growth Inhibition *(in vivo)*

Name of experiment	Drugs	Dose (mg/kg)	No. of EAC cells/mouse on day 5 after tumor cell inoculation	% of cell growth inhibition
Control	-	-	$9.72 \pm 1.72 \times 10^7$	-
Bleomycin	Antibiotic	0.3mg/kg	$0.60 \pm 0.98 \times 10^7$	93.8%
2-oxo benzylidine	Synthetic	16mg/kg	$0.43 \pm 1.47 \times 10^7$	95.6%
(3-oxo aniline) Cu(II)	Synthetic	8mg/kg	$0.61 \pm 1.32 \times 10^7$	93.9%
ethylenediamine	Synthetic	2mg/kg	$0.82 \pm 1.02 \times 10^7$	91.7%
complex	Synthetic	0.3mg/kg	$2.53 \pm 0.83 \times 10^7$	73.0%
Acryl hydroxamic	Synthetic	20mg/kg	$2.20 \pm 0.79 \times 10^7$	77.4%
acid				

Table 3: Effect of test compounds on survival time of EAC cells bearing mice.

Name of experiment	Extracts	Dose (mg/kg)	Mean survival time (Days) Mean ± SEM	Increase of life span %
Control	-	-	19.75 ± 2.8	-
EAC+ Bleomycin	Antibiotic	0.3	37.00 ± 1.52	87.34
2-oxo benzylidine (3-oxo aniline)		2	21.75 ± 2.4	10.12
Cu(II) ethylenediamine complex		8	25.00 ± 4.6	26.00
		16	28.5 ± 4.5	44.30

Table 4: Effect of 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex at different doses on average tumor weight.

Days	Control (EAC)	Bleomycin (0.3mg/kg i.p.)	2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex		
			2 mg /kg	8 mg /kg	16 mg /kg
0	0.00	0.00	0.00	0.00	0.00
2	0.75± 0.48	1.24± 1.5	0.725± 0.10	0.68± 0.26	0.7± 0.48
4	1.65± 0.7	0.58± 0.81	0.9± 0.56	0.61± 0.37	0.58± 0.43
6	3.13± 0.34	0.96± 0.56	1.0± 0.47	0.52± 0.52	0.46± 0.21
8	3.41± 0.41	1.5± 1.2	1.5± 0.31	0.40± 0.87	0.35± 2.5
10	4.0± 0.85	1.3± 0.4	2.0± 1.5	0.41± 0.78	0.30± .38
12	5.72± 0.72	2.1± 0.69	4.5± 2.1	0.65± 0.69	0.49± 0.85
14	6.88± 1.9	2.3± 0.16	6.0± 1.21	0.81± 0.98	0.76± 0.74
16	6.99± 2	3.01± 0.49	6.5± 0.39	0.80± 1.46	0.74± 0.62
18	8.20± 1.82	4.0± 1.8	7.0± 2.2	1.1± 1.37	0.98± 0.71
20	10.5± 2.1	4.56± 1.1	7.2± 2.4	1.6± 2.15	1.4± 0.50

No. of EAC cells 140×10^4 per ml were inoculated into 5 groups of mice (4 mice in each) on day 0. Four groups of mice were treated with the compound at the doses of 2, 8, 16mg/kg i.p. and bleomycin (0.3mg/kg i.p.) respectively after 24 hrs of EAC cell inoculation in mice and continued for 10days. Fifth group was considered as untreated control.

Table 5: Effect of 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex at the doses of 2 mg/kg, 8mg/kg and 50mg/kg on hematological parameters of normal and tumor bearing mice on day 12.

Name of The exp.	RBC cells/ml Mean	WBC/ml Mean	% of Hb Mean	Lymphocytes % Mean	Neautrophill % Mean	Monocytes % Mean
Normal	$8.3 \pm 0.57 \times 10^9$	$6.0 \pm 0.11 \times 10^6$	13.25± 0.4	71± 0.91	19± 2	9± 0.3
Control	$2.44 \pm 0.2 \times 10^9$	$26.7 \pm 0.41 \times 10^6$	8.25± 0.8	43± 0.75	38± 1.2	11± 0.6
EAC+ 2mg/kg	$2.9 \pm 0.48 \times 10^9$	$23.0 \pm 0.28 \times 10^6$	7.5± 0.33	49± 1.5	31± 0.92	8± 0.55
EAC+ 8mg/kg	$4.1 \pm 0.27 \times 10^9$	$20.7 \pm 0.17 \times 10^6$	9.87± 0.22	54± 1.1	29± 1.6	10± 0.8
EAC+ 16mg/kg	$6.5 \pm 0.67 \times 10^9$	$16.5 \pm 0.22 \times 10^6$	10.5± 0.14	56± 0.98	26± 2.1	8± 0.41
N+ 2mg/kg	$7.1 \pm 0.89 \times 10^9$	$10.0 \pm 0.12 \times 10^6$	11.0± 0.2	75± 1.85	20± 0.99	6± 0.5
N+ 8mg/kg	$5.6 \pm 0.72 \times 10^9$	$7.0 \pm 0.25 \times 10^6$	10.3± 0.9	68± 2	17± 0.81	8± 0.2
N+ 16mg/kg	$5.1 \pm 0.64 \times 10^9$	$8.0 \pm 0.5 \times 10^6$	11.25± 0.7	73± 0.78	22± 0.71	12± 0.61

N= Normal

Table 6: Effect of 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex at the doses of 2, 8 and 16mg/kg on serum alkaline phosphatase activity of normal and tumor bearing mice:

Name of experiment	Enzyme activity (μmol of PNPP hydrolyzed/min/ml serum)
Normal	$29.69 \pm 0.71 \times 10^{-3}$
Control	$8.63 \pm 0.39 \times 10^{-3}$
DMSO	$28.48 \pm 0.4 \times 10^{-3}$
EAC + Bleomycin(0.3mg/kg)	$27.36 \pm 0.76 \times 10^{-3}$
EAC + 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex (2mg/kg)	$8.83 \pm 0.9 \times 10^{-3}$
EAC + 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex (8mg/kg)	$13.33 \pm 0.29 \times 10^{-3}$
EAC + 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex (16mg/kg)	$21.81 \pm 0.41 \times 10^{-3}$
Normal + 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex (2mg/kg)	$26.36 \pm 0.62 \times 10^{-3}$
Normal + 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex (8mg/kg)	$28.33 \pm 0.75 \times 10^{-3}$
Normal + 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex (16mg/kg)	$47.05 \pm 0.8 \times 10^{-3}$

Effect of the test samples on cell growth inhibition: Effect of 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex, Acryl hydroxamic acid; Bleomycin on EAC cell growth on 5th day after tumor transplantation are shown in Table 2.

Treatment with 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex resulted in maximum cell growth inhibition at dose rates of 16mg/kg and 8mg/kg as evident from 95.6 & 93.8% reduction of tumor cells, which was found to be equivalent to standard antitumor agent bleomycin. For treatment with bleomycin, cell growth inhibition was 94.4%. 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex at the dose of 2mg/kg & 0.3mg/kg showed cell growth inhibition by 91.7 and 74% respectively.

Effect of the test compounds on survival time: Effect of 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex at different doses have been summarized in Table 3. It was found that treatment of the tumor induced test animals with the compound at the doses of 2mg/kg, 8mg/kg and 16mg/kg resulted in increase of life span by 10.12, 26 and 44.3% respectively when compared with control. Thus the survival time was to be increased when the dose of the compound was increased from 2 to 8mg/kg and to 16mg/kg in test animals (tumor bearing). It was found that the anticancer antibiotic bleomycin increase the life span by 87.3% when compared with control.

Effect of the test compound on body weight due to tumorigenesis in mice (average tumor weight): Effects of 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex at 2, 8 and 16mg/kg i.p. doses and the antibiotic bleomycin on the body weight due to tumorigenesis average tumor weight are shown in Table 4. Treatment of the test animals with test compound, previously inoculated with EAC cells, resulted in the inhibition of tumor growth. In the case of control (EAC) group, the body weight was increased by 48.24% in 20 days compared with the normal. By challenging the mice with 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex at the doses of 2, 8 and 16mg/kg; the body weight was increased by 16.8, 4 and 0% in 20 days due to the inhibition of tumor growth compared with 10% increase in the body weight of normal mice during the same period of time.

In contrast, the use of higher dose of the test compound (16mg/kg) dramatically inhibit the tumor growth in test animals near the antibiotic bleomycin (0.3mg/kg) used as standard (Table 4).

Effect of the test compound on hematological parameters in normal and tumor bearing mice on day 12: The growth of tumor in mice induced by EAC cells affected in acute anaemic condition as indicated by the significant decrease of the red blood cells and hemoglobin content determined on day 12 of tumor inoculation when compared with normal test animals under similar conditions (Table 5). The use of 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine complex at moderate dose (8mg/kg) partially and at high dose (16mg/kg) very nearly restored the red blood cell counts when compared with normal ranges. The total WBC count was also markedly decreased in the control group. Use of the test compound at low dose (2mg/kg) could not restor it significantly but high dose could (Table 5).

All these altered parameters in differential count of WBC were very nearly restored by the use of the test compound at 16mg/kg but partially restored at 8mg/kg (Table 5).

Effect of the test compound on hematological parameters of normal mice was not significant at both doses (Khanam and Masud Rana, 2001).

Effect of the test compound on serum alkaline phosphatase activity level in normal and tumor bearing mice on day 12: Effect of the test compound at the dose of 2, 8 and 16mg/kg on serum alkaline phosphatase activity determined on day 12 of tumor inoculation in normal and tumor bearing mice are summarized in Table 6.

Serum alkaline phosphatase activity level in tumor bearing mice was markedly decreased (70.5%) due to tumorigenesis when compared to the control mice.

Treatment with the test compound at 2mg/kg restored the enzyme activity at very low level partially (0.28%) but at 8mg/kg nearly restored (15.4%) and 16mg/kg very nearly restored (43.6%) the level, where the antibiotic bleomycin restored 68.3% of the depleted level (Table 6). Both doses of the compound on normal mice had no marked effects on serum alkaline phosphatase.

Considering the experimental results of tumor cell growth inhibition, survival time of tumor bearing mice, Hematological parameters and serum alkaline phosphatase activity in tumor bearing mice, it can be concluded that 2-oxo benzylidine (3-oxo aniline) Cu(II) ethylenediamine possess antineoplastic activity.

Acknowledgment

The authors are indebted to Mrs. Jahanara Khanam, Associate Professor, Department of Biochemistry, Rajshahi University, Bangladesh for providing the laboratory facilities.

References

Busch, H., 1974. The Molecular Biology of Cancer. Academic Press Inc., New York, pp: 329-340.

Clark, W.G., D.C. Brathers and A.R. Johnson, 1989. Goths Medical Pharmacology. 12th Edn., Galgotia Publications Pvt. Ltd., New Delhi, pp: 702-13.

Geary, W.J., 1971. The use of conductivity measurements in organic solvents for the characterization of coordination compounds. Coord. Chem. Rev., 7: 81-122.

Gilman, A.G., W. Rall and S.A. Nies, 1991. The pharmacological basis of therapeuties. Science, 2: 1211-1253.

Harder, H.C. and B. Rosenberg, 1970. Inhibitory effect of antitumor platinum compounds on DNA, RNA and protein synthesis in mammalian cells in vitro. Int. J. Cancer, 6: 207-216.

James, M., J.M. Orten and O.W. Neuhaus, 1995. Human Biochemistry. 9th Edn., C.V. Mosby, Saint Louis.

Khanam, J.A. and A.Y.K.M. Masud Rana, 2001. Antineoplastic activity of chloroacetohydroxamic acid in combination with bleomycin against ehrlich ascites carcinoma (EAC) in mice. J. Med. Sci., 1: 288-291.

Mycek, M.J., R.A. Harvey and P.C. Champe, 1997. Lippincott's Illustrated Reviews: Pharmacology. 2nd Edn., Liprincott-Raven, Philadelphia.

Power, C.B., 1981. Cell Biology. 2nd Edn., Plenum Press, New York.

Voet, D. and J.G. Voet, 1990. Biochemistry. 2nd Edn., John Wiley, New York.

Welsch, C.W., 1971. Growth inhibition of rat mammary carcinoma induced by Cis-platinum diaminodichloride-II. J. Natl. Cancer Inst., 47: 1071-1071.