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Synthesis, characterization and biological evaluation of a novel vanadium complex as a possible anticancer agent

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ABSTRACT

Chemotherapeutics are the sole treatment modality for cancer patients at advanced condition. In the pursuit of getting an efficient, selective and non toxic anticancer agent that has not been discovered yet, we rationally synthesized a vanadium complex with a Schiff base ligand *N*-(-2-hydroxyacetophenone) glycinate. Characterization of the synthesized complex $VO(NG)_2$, was carried out with the help of various spectroscopic means. The complex proved to be a broad spectrum anticancer agent with significantly less toxicity towards normal cells as evident from cell viability assay. Furthermore, $VO(NG)_2$ treatment increased the life-span of ascitic and solid tumor bearing Swiss albino mice and did not show any significant symptomatic sub acute toxicity on normal Swiss albino mice.

Introduction

Success of cancer chemotherapy relies on selective induction of cytotoxicity to transformed self cells, but such specific drug is elusive till date. Application of a transition metal complex, e.g., cisplatin as anticancer drug (ACD) showed us a new direction to fight against cancer [1,2]. Our laboratory has also participated in the crusade against cancer by introducing various transition metal complexes having the organic moiety *N*-(2-hydroxyacetophenone) glycinate (NG) as their ligand [3]. These metal chelates viz., CuNG, FeNG, ZnNG, MnNG imparts oxidative stress in the malignant cells by depleting cellular glutathione and thus induce apoptosis to the same [4–8]. Herein, we tried to explore the anticancer potential of a vanadium complex formed with the same ligand, NG. Vanadium; a trace element for various mammalian species has therapeutic applications to abrogate diabetes and AIDS [9,10]. Growing body of evidences discloses that vanadium complexes are potent anticancer agent but proves to be toxic at higher doses [11].

Deregulated cell cycle checkpoints is one of the pivotal hallmarks of cancer [12], and vanadium exerts its intracellular effect by inhibiting or degrading the protein tyrosine phosphatases (PTP's) which triggers apoptosis in neoplastic cells via cell cycle arrest [13,14]. Vanadium compounds also trigger apoptosis in malignant cells either by inducing proapototic genes or by inhibiting anti-apoptotic gene expression [15]. Contemporary studies reveal that transformed cells maintain an abnormal redox homeostasis that helps them to acquire optimal genetic instability, which is instrumental for cancer progression [16]. Vanadium being redox-active can generate reactive oxygen species (ROS). This heightened ROS disrupts the redox homeostasis in neoplastic cells and imparts redox stress leading to apoptosis either by cleaving DNA or by facilitating mitochondrial membrane permeabilization [17].

Under this scenario, antiproliferative effect of the synthesized complex $VO(NG)_2$ on a number of human and murine cancer cell lines were evaluated. The anticancer potential was further established on ascites and solid tumor bearing mice models. The subacute toxicity of the complex was also evaluated on normal mice.

Materials and methods

Reagents

3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), glycine, *N*-(2-Hydroxy) acetophenone, vanadyl sulphate were purchased from Sigma Chemical Company (St. Louis, USA). All

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other chemicals used were of highest purity and purchased from commercial sources.

Synthesis of the ligand, potassium N-(2-hydroxy acetophenone) glycinate (NG)

The ligand, potassium *N*-(2-hydroxy acetophenone) glycinate (NG) was prepared following the method of Majumder et al. as reported earlier [18]. In brief, KOH (18.39 mM) solution was added drop wise to glycine (18.4 mM) solution. The mixture was kept in an ice bath and stirred continuously at 15–20 °C. An ethanolic solution (10%) of 2 hydroxyacetophenone was added slowly to the mixture. Stirring was continued for 1 h following addition and the mixture was kept at room temperature for 5 h. The solvent was removed from the mixture (deep yellow) by a rotary evaporator. The yellow mass so obtained was washed with pet-ether and precipitated with methanol-diethyl ether mixture (1:1). The crude product was recrystallised from methanol. The pure crystallized NG deposited, yield 75%, m.p. 258–260 °C.

Synthesis of the vanadium complex of NG

Vanadium *N*-(2-hydroxy acetophenone) glycinate [$VO(NG)_2$] was synthesized from the ligand, potassium *N*-(2-hydroxy acetophenone) glycinate by its reaction with vanadyl sulphate. The aqueous solution of vanadyl sulphate (1 mM) was added to the aqueous solution of NG (1.1 mM) drop-wise. The solution was stirred magnetically at room temperature for 2 h and kept at 4 °C for 1 week. Brown crystalline precipitate was deposited from the solution and was filtered off and dried. Yield 67%, m.p. 191–193 °C. Anal. Calc. $C_{20}H_{20}O_7N_2V$: C, 53.21, H, 4.42, N, 6.2; Found: C, 52.46; H, 4.04; N, 5.9.

Chemical characterization and instrumentation

Elemental (C, H, N) analysis was performed with the help of Perkin–Elmer 2400 Series II CHN analyzer. UV–Vis spectra for the complex and the ligand were recorded in Varian Cary 100 Scan (range 800–200 nm) in dimethyl sulfoxide (DMSO). IR spectra were recorded in Perkin–Elmer RX 1 FT spectrophotometer in KBR discs, range 4000–400 cm^{-1}. Mass spectrum was recorded in Waters Micromass Quattro micro API mass spectrometer, in DMSO. ^1H NMR and ^{13}C NMR spectra of the complex were recorded at room temperature in a Bruker Avance 300 MHz spectrophotometer using 5 mm BBO probe in DMSO-d_6 solution. Thermogravimetric analysis was performed on a TA instrument (SDT Q600) thermal analyzer in a dynamic atmosphere of ultra high pure dinitrogen (flow rate: 100 cc min^{-1}). The sample was heated in platinum crucible at a rate of 10 °C min^{-1} from 25 °C to 900 °C. Cyclic voltametric study was done in BASi Epsilone EC instrument (USA). Electrodes used were Ag/AgCl (reference electrode), platinum auxilary electrode and platinum working electrode in DMSO using tetrabutylammonium hexafluorophosphate, [*N*-(*n*-Bu)4] PF_6; 0.1 M as supporting electrolyte at a scan rate of 100 mv/s (25 °C), reference against Ferrocinium/Ferrocene (Fc+/Fc). The complex solution was purged with N_2 for 30 min before each experiment.

Cell culture

The human T-cell acute lymphoblastic leukemia cell line CCRF-CEM (gifted by Prof T. Efferth, University of Mainz, Germany) and human colorectal carcinoma HCT-116 (ATCC) were maintained in RPMI 1640 medium (GIBCO Invitrogen Corp, Carlsbad, California, USA) supplemented with 10% foetal bovine serum (FBS) (GIBCO), glutamine (0.15%), HEPES (25 mM) and gentamycin (50 mg/ml). Furthermore the human breast cancer cell line MCF-7 and human astrocytoma (glioblastoma) cell line U373MG (NCCS, Pune) and mouse normal fibroblast cell line NIH 3T3 (provided by Dr. M. M. Gottesman, NIH, USA) were maintained in DMEM medium (GIBCO), supplemented with 10% FBS, additional glutamine (0.15%), HEPES (25 mM) and gentamycin (50 mg/ml). Cells were grown in plastic tissue culture flasks (Greiner Bio-One, Frickenhausen, Germany) in a 5% CO_2 atmosphere at 37 °C. Cells were passaged twice weekly. Cells from exponentially growing cultures of 15th passage were used for all experiments.

EAC (Ehrlich ascites carcinoma) and S180 (Sarcoma 180) cells were cultured *in vitro* by isolating the ascitic fluid from EAC or S180 bearing mice within 7–10 days of inoculation with the cancer cells. Cells were collected and washed thrice in PBS and then seeded accordingly in each well of flat bottomed 96-well plate for MTT assay with RPMI 1640 (for EAC) or DMEM (for S180) medium (GIBCO), containing HEPES (Sigma), penicillin–streptomycin and 10% FBS.

Isolation of PBMC

Heparinized human blood (peripheral) was collected and diluted with RPMI 1640 (1:1, v/v). The Lymphocyte-enriched mononuclear cell layer [Peripheral Blood Mononuclear Cells (PBMC)] were then isolated with the aid of Histopaque 1077 (Sigma). The procured cells were washed and finally resuspended in cold RPMI 1640 with above-mentioned supplements.

Treatment

For in vitro assay a solution of $VO(NG)_2$ (1 mg/ml) was prepared just before experiments by dissolving the lyophilized compounds in DMSO (0.01%) following serial dilution in FBS containing medium to reduce the concentration of DMSO to nontoxic level i.e. less than 0.01%. For MTT assay, $VO(NG)_2$ treatments were performed with a concentration range from 2 to 28 μg/ml. For *in vivo* experiments a solution of $VO(NG)_2$ (1 g/ml) was prepared just before experiments by dissolving the lyophilized compounds in DMSO (0.01%) following serial dilution in PBS containing medium to reduce the concentration of DMSO to nontoxic level i.e. less than 0.01%).

In vitro viability assay (MTT assay)

Cell viability was determined by the method as described previously [19]. In brief, cells were seeded in 96-well plates at a density 4×10^4 of cells per well. For single-agent studies, cells were seeded and allowed to acclimatize for 24 h before treatment with increasing concentrations of $VO(NG)_2$ and was incubated for further 48 h or 72 h with 5% CO2 at 37 °C. Following completion of incubation cells were further incubated with 5 mg/ml of MTT dye for 4 h at 37 °C. The cells were suspended in DMSO (0.1 ml) and the absorbance was read at 540 nm in an ELISA reader (Tecan 200) and the control value corresponding to drug untreated (solvent-treated) cells was taken as 100%. The viability of treated samples was expressed as percent of control. The IC_{50} values were determined as the concentration of $VO(NG)_2$ that reduced cell viability by 50% compared to control cells.

Animals

Swiss albino mice (originally obtained from National Institute of Nutrition, Hyderabad, India and reared in the institute animal facilities) were used for all the experiments with prior approval (Approval ID: IAEC-1.2/SKC-7/2007/4 dated 22.01.2008) of the institutional animal ethics committee (IAEC). The experimental protocols described herein were in accordance with the IAEC (Registration No.: 175/99/CPCSEA, dated 28.01.2000) guidelines laid down by the committee for the purpose of control and

supervision of experiments on animals (CPCSEA) by the Ministry of Social Justice and Empowerment, Government of India. Adult female Swiss albino mice (weighing 18–20 g, 4–6 weeks old) were acclimatized to the experimental room having temperature 25 ± 2 °C, controlled humidity conditions, and with photo cycle of 12 h light/12 h dark. The mice were housed in sterile polypropylene cages containing sterile paddy husk as bedding material, fed on autoclaved standard mice food pellets and water *ad libitum*.

Cell line, tumor transplantation and experimental protocol

Both EAC and S180 cells were maintained as an ascitic tumor in female Swiss albino mice. Various groups (each group containing six mice) of animals were taken for animal survival studies. Each mouse was inoculated with either 10^6 EAC cells intraperitoneally (i.p), or 10^6 S180 cells subcutaneously (on the right hind leg quarters) to develop ascitic or solid tumor respectively. Following 9 days inoculation, various $VO(NG)_2$ doses (20, 30, 40, 50 mg/kg of body weight) as well as vehicle control (DMSO) were administered i.p. to EAC bearing and intratumorally (i.t.) to S180 bearing mice groups. Various $VO(NG)_2$ doses (5, 10, 15 mg/kg body weight) and vehicle control (DMSO) were given intramuscularly (i.m.) to S180 bearing mice groups, while 1 group each from EAC and S180 inoculated individuals were kept as untreated control (Each experimental EAC bearing mice groups were kept in duplicate). Animals were inspected daily for the assessment of ascitic load, and body weights were measured on every 3rd day. Two weeks (at 15th day) after drug treatment, one set from the EAC bearing experimental groups of mice were sacrificed to measure the total ascitic fluid (TAF) and packed cell volume (PCV) while the other set was kept to record their mean survival time (MST). The MSTs for each group of animals were documented by noting the time of death of each mice. The MST of each experimental group was compared with the MST of untreated control group to obtain the treated/control (T/C) ratio (percent) for every treated groups separately. This T/C value for each experimental group is instrumental for the *in vivo* validation of the effect of $VO(NG)_2$ on animal survival. National Cancer Institute (NCI, USA) considers the T/C ratios around 120% to be "marginal", between 120 and 150% as "clear" and equal or superior to 150% as "marked" in terms of anticancer activity [20].

In vivo toxicity assay for $VO(NG)_2$

To determine the sub-acute toxicity of $VO(NG)_2$, different experimental groups of normal female Swiss albino mice (6 mice in each group) were treated i.p with various doses of $VO(NG)_2$ (20, 30, 40 and 50 mg/kg body weight). Some animals were treated with $VO(NG)_2$ (5, 10 and 15 mg/kg body weight) through i. m. route Appropriate vehicle control was maintained by injecting the animals with DMSO through i.p. and i.m. routes. Some animals were also kept untreated as control. Following 14 days treatment, blood was collected via closed cardiac puncture with the help of a 22-guage hypodermic needle [21]. Blood from each group was then pooled into separate glass tubes and treated with anticoagulant (heparin) or left untreated for serum collection. Hematological parameters were then analyzed by automated haematology analyzer (Sysmex, KX-21) and hematologic biochemical analyses (blood urea, creatinin, alkaline phosphatase, ALT, AST) was performed by the help of automated clinical chemistry analyzer (Olympus, AU400).

Isolation of bone marrow cell

Preliminary bone marrow toxicity was assayed following administration of various doses of $VO(NG)_2$ [(i.p; 20, 30, 40, 50 mg/kg body weight) and (i.m; 5,10,15 mg/kg weight)] on female Swiss albino mice.14 days after the treatment the experimental mice were anaesthetized and the femur bone was cut with the help of a vertebrate scissor. Bone marrow was flushed with KCl solution (0.56%) and centrifuged at 3000 rpm for 15 min at 37 °C. Cell viability was tested by trypan blue solution (0.4%) and cells were counted in a phase contrast microscope. The experiment was repeated thrice. The average value of viable bone marrow cells was calculated (percentage) from three independent experiments.

Isolation of spleen cell

Preliminary spleenic toxicity (sub-acute) of $VO(NG)_2$ treated [(i.p; 20, 30, 40, 50 mg/kg body weight) and (i.m; 5,10,15 mg/kg weight)] female Swiss albino mice was further investigated keeping appropriate vehicle and untreated control. Mice of different groups were anaesthetized and 70% alcohol was sprayed on abdominal region. Spleen was removed aseptically and small amount of PBS was injected to it; Spleen was rubbed against the fine wire mesh of the tissue grinder. The cell suspension formed is spun at 1000–1500 rpm for 5–10 min. The supernatant was discarded and the cells were washed by spinning in PBS twice at room temperature. Cell viability was tested by trypan blue solution (0.4%) and cells were counted in a phase contrast microscope. The experiment was repeated thrice .The average value of viable spleen cells was calculated from three independent experiments.

Statistical analysis

The values of the arithmetic mean from three independent experiments performed in triplicate, i.e., mean ± S.D. are reported in the present communication. The unpaired Student's *t*-test was used to evaluate the significance of differences between treated versus control groups, accepting $P < 0.05$ as a level of significance. The data were analyzed using the Prism software (GraphPad, San Diego, CA).

Results

UV–VIS spectral study

UV bands for the ligand appeared at λ max (DMSO): 318, 347 and 423.

UV bands for the complex appeared at λ max (DMSO): 231, 264 and 326.

The change in the UV peak from 318 in the ligand to 231 in the complex indicates $\pi-\pi^*$ transition. The shift of the peak from 347 in the ligand to 264 in the complex also indicates $\pi-\pi^*$ transition. The bathocromatic shift of the peak from 423 in the ligand to 326 in the complex also indicates $\pi-\pi^*$ transition.

IR spectrum

Important infrared (IR) bands for the ligand appear at: 3410–3360, 1689, 1619, 1524, 1466, 1421, 1395, 1318, 1269, 1205, 1163, 969, 931, 752 and 730 cm^{-1}.

Important IR bands for the complex appear at: 1645, 1609, 1443, 1379, 1324, 1252, 1131, 982, 936, 834, 745 and 436 cm^{-1}.

The IR spectrum of the ligand showed broad band at 3360–3410 cm^{-1} indicating the presence of phenolic ν_{OH} group. The free ν_{OH} is generally observed between 3400 and 3500 cm^{-1}. The observed low value is due to intramolecular H-bond formation between H and nitrogen [18]. In vanadium complex the band disappeared and thus indicating complexation involving phenolic –OH group of the ligand. The alkyl groups CH_2, CH_3 show characteristic deforming bands at 1466–1395 cm^{-1} and the rocking modes at 730 cm^{-1} in the ligand. In complex the deforming bands and rocking

modes are shifted to 1443 cm^{-1} and 745 cm^{-1} respectively. The characteristic imine band at 1619 cm^{-1} (ligand) is shifted towards 1609 cm^{-1} further conforming complex formation. The sharp band at 1395 cm^{-1} in the ligand may be assigned due to ν_{OCO} symmetric stretching that has been shifted to 1379 cm^{-1} in the complex and thus indicating coordination with the V atom through the $-COO^-$ group of the ligand. The band at 1269 cm^{-1} in the ligand may be assigned due to $\nu_{Ph(CO)}$ which has been shifted to 1252 cm^{-1} in the complex indicating coordination of the phenolic oxygen to the VO moiety. The band at 1689 cm^{-1} in the ligand may be assigned due to a $\nu_{(OCO)}$ asymmetric vibration and in complex this vibration appears as broad band at 1646 cm^{-1} [22]. Two new bands only in the complex at 982 cm^{-1} and 436 cm^{-1} confirm the presence of vanadyl and V—N functionality respectively [23,24] The sharp peak in the spectra of the complex at 1609 cm^{-1} is characteristic of the imine group and the band at 1443 cm^{-1} is due to skeletal vibration of the aromatic moiety. These bands were observed at a relatively lower frequency than in ligand NG which is indicative of complex formation. Thus, the IR spectra results provide strong evidence for the complex formation.

Mass spectral study

Mass spectral data is presented in Fig. 1. The formation of molecular ion peaks indicates that the structure of the complex is A in Fig. 1.

^{1}H NMR analysis

^{1}H NMR of $VO(NG)_2$ (DMSO-d_6, 300 MHz, 300 K): δ 7.8 (d, 1H, $J = 0.028$), 7.4 (t, 1H, $J = 0.025$), 6.8 (dd, 2H, $J = 0.018, 0.026$), 3.527 (s, 2H), 2.51 (s, 3H).

^{13}C NMR analysis

^{13}C NMR of $VO(NG)_2$ (DMSO-d_6, 300 MHz, 300 K): δ 157.8 (C-1), 116 (C-2), 133 (C-3), 123 (C-4), 129 (C-5), 119 (C-6) 164 (C-7), 19.2 (C-8), 57 (C-9), 171 (C-10).

The peaks appeared at 116-133 had been assigned for the aromatic carbons. The peaks for —COOH carbon appeared at 171. The peak for —CH=N carbon, $=CH_2$ carbon and $-CH_3$ carbon were noted 164, 57 and 19 respectively in the spectrum (Supplementary material). The ^{13}C NMR peaks were in agreement with the skeletal structure of $VO(NG)_2$.

Thermogravimetric analysis of $VO(NG)_2$

Thermogravimetric analysis of complex, $VO(NG)_2$, have been performed to elucidate the solid state thermal behavior, to indentify the end products and thereby the composition of the original species. The analysis revealed that upon gradual heating the complex decomposes in two steps. The first step occurs at ~220 °C is probably due to a partial decomposition of the ligand (observed 33%) (calculated weight loss for 1 molecule of ligand NG: 42.57%). The second step of the thermal decomposition, which occurs at ~410 °C, was assigned to the loss of the second ligand molecule (observed 82%) (calculated.85.14%) with the formation of VO_2 (observed 19%) (calculated.18.40%).

Cyclic voltammetry analysis

Cyclic voltammetric assay of $VO(NG)_2$ was performed to find out whether it has the ability to alter cellular redox status. The

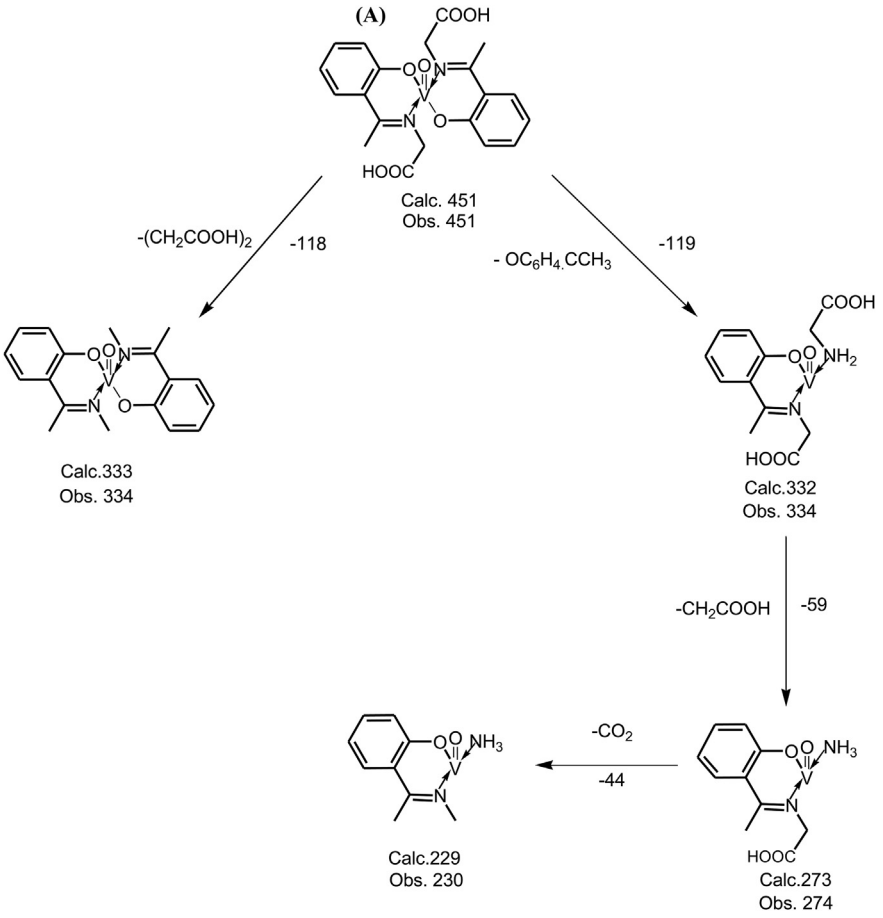

Fig. 1. Mass spectral analysis. Mass fragmentation pattern of $VO(NG)_2$, depicting the chemical structure of the complex (A).

compound exhibits irreversible voltammogram with an oxidation peak at 0.150 V and reduction peak at −0.348 V. The result shows that the compound possibly generates ROS at very low volt (−0.348 V); thus the compound has very low reduction potential and is capable of generating ROS in physiological condition.

$VO(NG)_2$ exhibits antiproliferative effect selectively on malignant cell lines in time and dose dependent manner

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was conducted on a number of $VO(NG)_2$ treated malignant cells such as, MCF 7, HCT 116, U373 MG, CCRF-CEM, EAC, Sarcoma 180, human normal PBMC and NIH 3T3 (Fig. 2).

Our data clearly indicated that $VO(NG)_2$ exhibits time and dose dependent antiproliferative activity on the above mentioned cancer cells (Table 1). But under the influence of identical $VO(NG)_2$ concentrations we noticed miniscule inhibition of proliferation of normal human PBMC (Fig. 2) and no significant cytotoxicity on NIH 3T3 cells (Fig. 2). Furthermore, administration of the ligand (NG) to all the malignant cells herein at identical doses imposed negligible cytotoxicity.

Table 1
Comparative account of antiproliferative effect of $VO(NG)_2$ on various cells.

Cell line	IC_{50} dose (μg/ml)	Growth inhibition (%) at highest conc. of $VO(NG)_2$
HCT 116	–	9.9 ± 2.7
MCF-7	1.51 ± 0.11	57.57 ± 7.9
U373MG	28 ± 1.9	79.79 ± 3.4
CCRF-CEM	17.10 ± 0.93	82.82 ± 4.1
S180	12.21 ± 0.84	74.98 ± 7.0
EAC/S	–	47.86 ± 5.4
Normal human PBMC	–	11.94 ± 0.68
NIH3T3	–	–

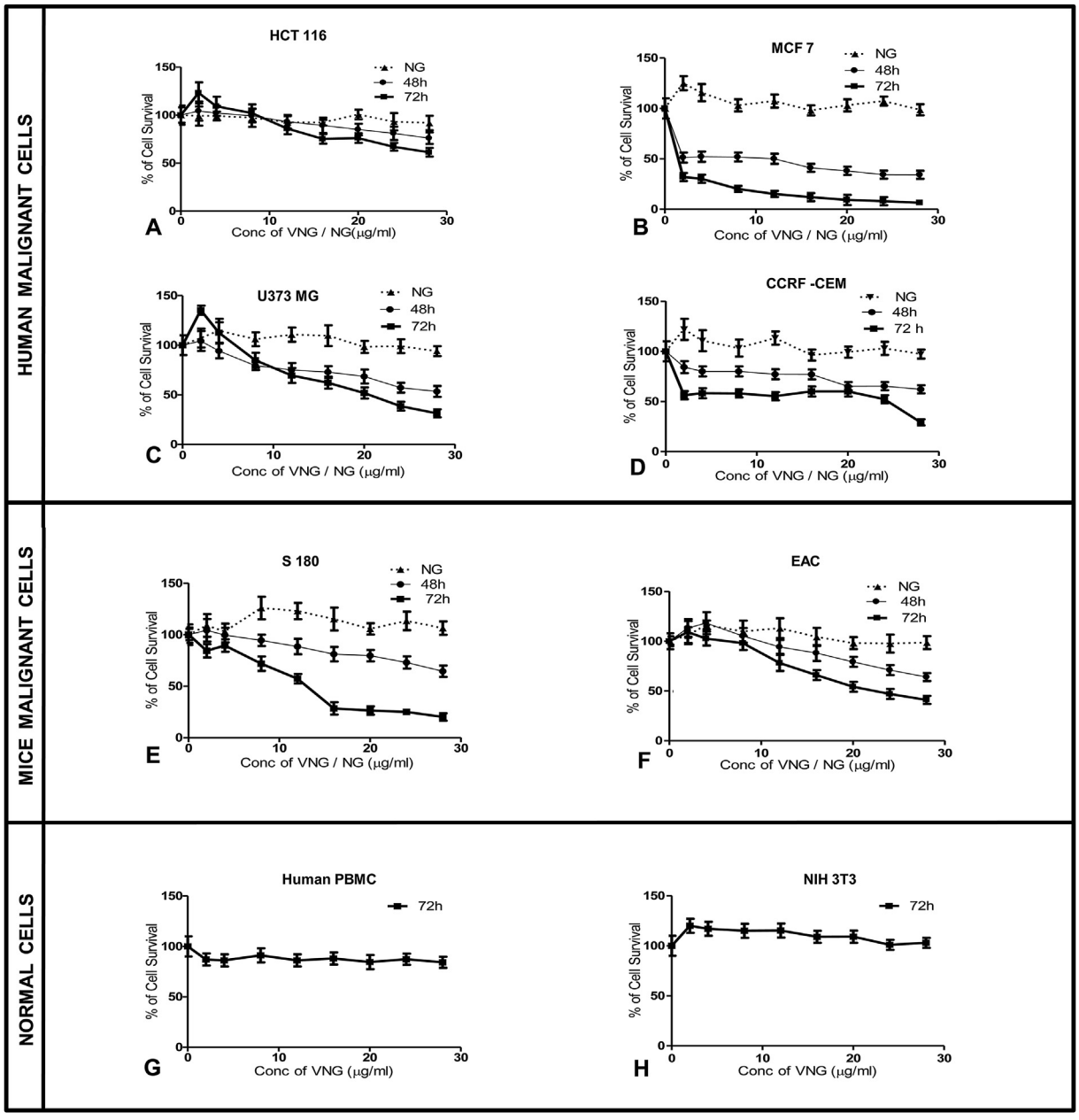

Fig. 2. Comparison of the cytotoxic effect of the vanadium complex and its ligand on different cells.

Dose response curves prepared for vanadium complex ($VO(NG)_2$) and the ligand (NG) on (A) HCT116, (B) MCF7, (C) U373MG, (D) CCRF-CEM, (E) S180, (F) EAC cell lines and $VO(NG)_2$ alone on (G) Human PBMC and (H) NIH3T3 cells according to MTT assay. All the cancer cells herein were treated with similar doses of NG and was incubated further for 72 h. Results are expressed as percentage viability of treated cells compared to control (solvent-treated) cells. Value represents the mean ± SD of three independent experiments with four replicates in each (Fig. 2).

Survivability assay of $VO(NG)_2$ treated cell lines showing their respective IC_{50} values and or survivability of cells receiving 28 μg/ml $VO(NG)_2$ (72 h incubation). Results are expressed as percentage viability of treated cells compared to control (solvent-treated) cells. Values represent the mean ± SD of 3 independent experiments with 4 replicates in each (Table 1).

$VO(NG)_2$ imparts no significant toxicity on female Swiss albino mice

A preliminary 14 days (sub-acute) toxicity study was carried out on normal female Swiss albino mice model receiving $VO(NG)_2$ doses either i.p. (20, 30, 40 or 50 mg/kg body weight) or i.m. (5, 10, 15 mg/kg body weight). Herein we observed that no significant toxic death occurred following vehicle (DMSO) and $VO(NG)_2$ treatment. Hematological (Table 2) and other biochemical parameters that are indicators for hepatic and renal toxicity (Table 3) remained unchanged following $VO(NG)_2$ application (up to highest dose of 50 mg/kg body weight) compared to untreated control. Furthermore no spleen and bone marrow toxicity in vehicle (DMSO) as well as $VO(NG)_2$ treated groups compared to control was noticed (Table 4).

$VO(NG)_2$ (all the doses) through both i.p and i.m routes showed no hematological toxicity to normal female Swiss albino mice in comparison to untreated control mice ($P < 0.05$). Herein vehicle control (VC) is solvent (DMSO)-treated normal female Swiss albino mice. Values represent mean ± standard deviation (S.D.) from 3 independent experiments for 6 mice in each group (Table 2).

$VO(NG)_2$ (all the doses) through both i.p and i.m routes showed no hepatic and renal toxicity over normal female Swiss albino mice as compared with untreated control mice ($P < 0.05$). Herein vehicle control (VC) is solvent (DMSO) treated normal female Swiss albino mice. Values represent mean ± standard deviation (S.D.) from 3 independent experiments for 6 mice in each group (Table 3).

$VO(NG)_2$ (all the doses) through both i.p. and i.m routes were non-toxic to spleen and bone marrow cells of treated normal female Swiss albino mice when compared with untreated control mice ($P < 0.05$). Herein vehicle control (VC) is solvent (DMSO) treated normal female Swiss albino mice. Values represent mean ± standard deviation (S.D.) from 3 independent experiments for 6 mice in each group (Table 4).

Table 3
Effect of $VO(NG)_2$ on toxicological parameters for hepatic and renal function in female Swiss albino mice blood.

Groups	Urea (mg/dL)	Creatinine (mg/dL)	Alkaline phosphatase (U/L)	SGOT[a] (U/L)	SGPT[b] (U/L)
Untreated	54 ± 1.9	0.4 ± 0.01	200 ± 6.9	182 ± 5.9	53 ± 1.6
I.P (VC)	57 ± 1.9	0.3 ± 0.02	181 ± 6.1	183 ± 5.6	41 ± 0.9
I.P (20 mg/kg)	55 ± 1.4	0.3 ± 0.019	259 ± 7.6	170 ± 4.8	66 ± 1.9
I.P (30 mg/kg)	47 ± 1.0	0.4 ± 0.023	239 ± 7.1	188 ± 5.8	62 ± 2.1
I.P (40 mg/kg)	49 ± 2.1	0.3 ± 0.026	242 ± 6.7	196 ± 6.2	71 ± 2.4
I.P (50 mg/kg)	53 ± 1.4	0.4 ± 0.011	271 ± 7.7	189 ± 5.9	68 ± 2.1
I.M (VC)	51 ± 1.0	0.4 ± 0.021	193 ± 5.9	188 ± 5.7	56 ± 1.5
I.M (5 mg/kg)	43 ± 01.7	0.2 ± 0.013	176 ± 5.5	168 ± 6.0	47 ± 1.3
I.M (10 mg/kg)	52 ± 1.6	0.4 ± 0.03	187 ± 6.3	178 ± 5.3	56 ± 1.6
I.M (15 mg/kg)	59 ± 1.5	0.3 ± 0.017	204 ± 6.7	181 ± 5.5	61 ± 1.9

[a] Serum glutamate ortho-transferase (SGOT).
[b] Serum glutamine pyruvate transaminase (SGPT).

Table 4
Effect of $VO(NG)_2$ on spleen and bone marrow of female Swiss albino mice.

Groups	Bone marrow cell survival (%)	Spleen cell survival (%)
Untreated	95.3 ± 3.2	97.2 ± 3.8
I.P (VC)	96.2 ± 3.2	90.8 ± 2.5
I.P (20 mg/kg)	91.1 ± 2.7	93.4 ± 3.2
I.P (30 mg/kg)	92.7 ± 2.9	91.7 ± 2.6
I.P (40 mg/kg)	84.1 ± 2.8	90.3 ± 3.0
I.P (50 mg/kg)	83.5 ± 1.7	87.5 ± 2.5
I.M (VC)	93.2 ± 3.1	94.3 ± 3.7
I.M (5 mg/kg)	92.7 ± 2.8	93.2 ± 2.8
I.M (10 mg/kg)	91.3 ± 2.3	94.5 ± 3.6
I.M (15 mg/kg)	90.5 ± 2.5	89.7 ± 2.8

$VO(NG)_2$ enhanced the life-span of various malignant cell bearing mice

$VO(NG)_2$ found to be cytotoxic against MCF 7 (*in vitro*) and EAC cells (*ex vivo*). *In vivo* experimentation of this complex on EAC (originated as murine breast cancer) bearing mice model also exhibited anticancer potential. Out of all the doses, 30 mg/kg body weight of $VO(NG)_2$ when administered (i.p) to EAC bearing mice proved to be most effective showing a T/C value of 150%. The intratumoral (i.t.) efficacy of the complex was evaluated in S180 tumor bearing mice. However, VO(NG)2 administration through i.t. route proved to be less effective in S180.

Application of $VO(NG)_2$ through i.m. route in S180 bearing mice caused an abrupt increase in lymphocyte population compared to untreated control. (VONG)2 failed to modulate the lymphocyte population through i.m. route in EAC bearing mice (data not

Table 2
Effect of $VO(NG)_2$ on hematological parameters of female Swiss albino mice.

Parameters	(UT)	I.P VC	I.P (20 mg/kg)	I.P (30 mg/kg)	I.P (40 mg/kg)	I.P (50 mg/kg)	I.M VC	I.M (5 mg/kg)	I.M (10 mg/kg)	I.M (15 mg/kg)
WBC ($\times 10^3/\mu L$)	19.1 ± 1.3	19.0 ± 1.6	13.2 ± 0.9	12.4 ± 0.8	10.3 ± 0.8	18.2 ± 1.6	19.9 ± 1.2	22.9 ± 2.4	20.5 ± 1.9	17.1 ± 1.1
RBC ($\times 10^6/\mu L$)	9.65 ± 0.9	10.0 ± 0.9	9.17 ± 0.8	9.58 ± 0.9	6.20 ± 0.4	7.08 ± 0.8	9.06 ± 0.8	8.89 ± 0.7	9.02 ± 0.9	7.71 ± 0.5
HGB (g/dL)	15.0 ± 1.1	15.7 ± 1.1	14.0 ± 1.0	15.2 ± 1.1	10.0 ± 0.7	10.8 ± 0.8	14.2 ± 0.9	13.4 ± 1.1	13.5 ± 0.8	10.5 ± 0.9
HCT[a] (%)	49.3 ± 3.2	51.9 ± 3.9	46.6 ± 3.1	50.8 ± 3.8	34.6 ± 2.3	35.9 ± 1.9	46.6 ± 3.2	44.2 ± 2.8	46.3 ± 2.9	36.0 ± 2.1
MCV[b] (fL)	51.1 ± 3.8	51.9 ± 3.7	50.8 ± 3.6	53.0 ± 3.2	55.6 ± 3.1	50.7 ± 3.4	51.7 ± 3.9	49.7 ± 3.8	51.7 ± 3.5	52.3 ± 2.9
MCH[c] (pg)	15.5 ± 0.8	15.7 ± 0.7	15.3 ± 0.8	15.9 ± 0.9	16.1 ± 1.2	15.3 ± 0.7	15.6 ± 0.8	15.0 ± 0.9	14.9 ± 0.5	13.6 ± 0.5
MCHC[d] (g/dL)	30.4 ± 2.3	30.3 ± 2.1	30.0 ± 2.2	29.9 ± 2.0	28.7 ± 1.9	30.1 ± 2.3	30.9 ± 2.1	30.3 ± 2.1	28.9 ± 2.6	29.1 ± 2.4
PLT ($\times 10^3/\mu L$)	921 ± 38.8	1060 ± 31.4	1303 ± 41.1	1205 ± 36.0	1300 ± 37.4	1447 ± 44.3	1049 ± 29.7	1063 ± 40.2	1209 ± 35.2	922 ± 36.4
LYM (%)	88.5 ± 7.3	86.0 ± 7.1	88.4 ± 7.4	87.2 ± 7.2	74.1 ± 6.7	80.3 ± 6.9	87.4 ± 7.3	93.7 ± 8.1	88.3 ± 7.9	81.5 ± 2.1

[a] Hematocrit value (HCT).
[b] Mean corpuscular volume (MCV).
[c] Mean corpuscular hemoglobin (MCH).
[d] Mean corpuscular hemoglobin concentration (MCHC).

shown). Thus $VO(NG)_2$ may have the host protective immune modulatory potential on S180 bearing mice model. The result inspired us to apply the complex through i.m route into the S180 cancer model. Our data found accordance with the above mentioned hypothesis as $VO(NG)_2$ treatment (i.m.; 5 mg/kg and 10 mg/kg body weight) increased the survival of S180 bearing mice with T/C values of 150% and 162% respectively (Table 5).

$VO(NG)_2$ (various doses) through intraperitonel (i.p.), intratumoral (i.t.) and intramuscular (i. m.) routes increases the mean survival time (MST) and therefore T/C value of EAC and S180 cells bearing mice groups compared to untreated (control) groups. The packed cell volume (PCV) and the total ascitic fluid (TAF) were also decreased in treated groups compared to untreated EAC cell bearing mice. Herein vehicle control (VC) is solvent (DMSO)-treated mice. Values represent mean ± standard deviation (S.D.) from 3 independent experiments for 6 mice in each group (Table 5).

Discussion

The present work is based on our proposition that a coordination complex of vanadium may be less toxic and potent anticancer agent if a suitable organic moiety can be attached to vanadium atom [25]. Under this backdrop we selected the non-toxic ligand, NG which showed anticancer activity when bound to divalent copper, iron, manganese and zinc atoms [3]. We synthesized and characterized $VO(NG)_2$ and studied it's *in vitro* and *in vivo* anticancer potential. For *in vitro* studies we selected six transformed cell lines viz., human colorectal carcinoma (HCT 116), human breast carcinoma (MCF 7), human astrocytoma (U 373 MG), human T lymphoblastic leukemia (CCRF-CEM), murine Ehrlich ascites carcinoma (EAC) and murine sarcoma (S 180) models to determine whether $VO(NG)_2$ has differential antiproliferative effects on them or not. Our studies reveled that $VO(NG)_2$ inhibits the proliferation of all the above mentioned cell lines differentially, in time and dose dependent manner (Table 1). Furthermore, application of identical $VO(NG)_2$ dosage to normal human PBMC and normal murine NIH 3T3 cells did not hamper their survivability (Table 1). $VO(NG)_2$ proved to be most effective on MCF7 cell line with IC_{50} value as low as 1.60 ± 0.53 μg/ml (at 72 h of incubation) and also significantly cytotoxic to EAC cells (originated as murine breast cancer) (*ex vivo*). Thus, we selected EAC bearing Swiss albino mice as our *in vivo* model of interest. The S180 bearing Swiss albino mice were included for the present work to serve as a solid tumor bearing sarcoma model. Our study disclosed that $VO(NG)_2$ administration (i.p.) (30 mg/kg body weight) on EAC bearing mice exhibited T/C value of 150% whereas $VO(NG)_2$ administration (i.m) at lower doses (5 and 10 mg/kg body weight) proved to be most potent on S180 bearing mice (T/C values of 149% and 162.2%). All these doses showed "marked activity" according to standard National Cancer Institute protocols for screening new anticancer drugs [20]. Moreover, $VO(NG)_2$ treatment up to 50 mg/kg of body weight exhibited no significant toxicity on normal female Swiss albino mice.

Table 5
Effect of $VO(NG)_2$ on cancer bearing female Swiss albino mice.

Group	Route	Total ascitic fluid (ml)	Packed cell volume (ml)	Mean survival time (days)	T/C value (%)
EAC (UT)	–	13.5 ± 1.7	8.0 ± 1.2	22 ± 1.7	100
EAC (VC)	I.P	13.9 ± 1.8	8.3 ± 1.3	23 ± 1.8	104.5
EAC (20 mg/kg)	I.P	11.5 ± 1.4	6.2 ± 0.6	25 ± 2.0	113.63
EAC (30 mg/kg)	I.P	7.9 ± 0.9	4.7 ± 0.5	33 ± 2.7	150
EAC (40 mg/kg)	I.P	12.7 ± 1.6	7.6 ± 0.7	21 ± 1.7	95.4
EAC (50 mg/kg)	I.P	13.3 ± 1.5	7.9 ± 0.9	20 ± 1.8	90.9
S 180 (UT)	–	–	–	53 ± 4.3	100
S 180 (VC)	I.T	–	–	56 ± 4.1	105.6
S 180 (20 mg/kg)	I.T	–	–	53 ± 3.8	100
S 180 (30 mg/kg)	I.T	–	–	68 ± 5.2	128.3
S 180 (40 mg/kg)	I.T	–	–	59 ± 4.0	111.3
S 180 (50 mg/kg)	I.T	–	–	47 ± 3.5	88.6
S 180 (VC)	I.M	–	–	54 ± 4.7	101.8
S 180 (5 mg/kg)	I.M	–	–	86 ± 7.1	162.2
S 180 (10 mg/kg)	I.M	–	–	79 ± 6.7	149
S 180 (15 mg/kg)	I.M	–	–	64 ± 5.1	120.7

Our group previously reported numerous transition metal complexes viz, CuNG, FeNG, ZnNG, MnNG and all of these complexes alters redox homeostatic machinery of cancer cells [3–8]. $VO(NG)_2$ being a transition metal complex exhibits irreversible reduction at physiological electro chemical potential range as evident from cyclicvoltammetric analysis. Therefore, $VO(NG)_2$ probably imparts redox stress to the cancer cells by deregulating their redox homeostatic biochemistry in *in vitro* as well as *in vivo* cancer models. We also cannot rule out the possibility of ROS-mediated immune-modulation in the S180 tumor microenvironment following $VO(NG)_2$ (i.m) administration [26,27]. Thus in agreement with our proposition, the *in vitro* and *in vivo* studies indicate the promise of a broad spectrum anticancer agent in the form of present vanadium complex, $VO(NG)_2$.

Conclusions

This novel complex of vanadium, $VO(NG)_2$ proved to be a potent cytotoxic agent specifically for the malignant cells *in vitro* as revealed by cell survival assay. $VO(NG)_2$ also proved to be an effective antineoplastic agent *in vivo* on both ascitic (EAC) as well as solid tumor (S180) bearing female Swiss albino mice. However, $VO(NG)_2$ (5 mg/kg) when administered through i.m. route proved to be most potent on S180 bearing mice with a 'marked' T/C value of '162.2%'. Although vanadium is toxic at higher doses, surprisingly $VO(NG)_2$ treatment up to 50 mg/kg body weight exhibited no significant manifestation of toxic symptoms on female Swiss albino mice. As our preclinical data sufficiently justified our hypothesis, further efforts are devoted to understand the molecular mechanism of $VO(NG)_2$ induced cancer cell death.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jorganchem.2014.08.032.

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