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Preclinical report

Apoptosis-inducing oxovanadium(IV) complexes of 1,10-phenanthroline against human ovarian cancer

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In a systematic effort to identify a potent anticancer agent against human ovarian cancer, we synthesized 15 oxovanadium(IV) complexes, and examined their cytotoxic activity against human ovarian cancer cell lines PA-1, SKOV-3, ES-2 and OVCAR-3 using a MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyletetrazolium bromide]-based assay. The apoptosis-inducing ability of the oxovanadium compounds was evaluated by the two-color flow cytometric terminal deoxynucleotidyl transferase-based assay that labels 3′-hydroxyl ends of fragmented DNA (TUNEL) assay and confocal laser scanning microscopy. Notably, all eight oxovanadium complexes of 1,10 phenanthroline exhibited significant cytotoxicity and induced apoptosis within 24 h. The *mono*-chelated, $VO(NO_2\text{-phen})$ and *bis*-chelated, $VO(Me_2\text{-phen})_2$, $VO(Cl\text{-phen})_2$ and $VO(NO_2\text{-phen})_2$ complexes were the most potent oxovanadium compounds, and killed target cancer cells at low micromolar concentrations. The marked differences in the cytotoxic activity of oxovanadium(IV) complexes containing different heterocyclic ancillary ligands suggest that the cytotoxic activity of these compounds is determined by the identity of the five-member bidentate ligands, as well as the nature of the substituents on the heterocyclic aromatic rings. Our results presented herein provide experimental evidence that oxovanadium compounds induce apoptosis in human ovarian cancer cells. The lead compounds, $VO(Me_2\text{-phen})_2$ and $VO(NO_2\text{-phen})_2$, may be useful in the treatment of ovarian cancer. [© 2000 Lippincott Williams & Wilkins.]

Key words: Cisplatin, ovarian cancer, oxovanadium complexes, vanadium.

Introduction

Ovarian cancer remains the deadliest gynecologic malignancy in the US, with 14 800 deaths and 26 700 new cases estimated in 2000.[1] Approximately one in 70 women will develop ovarian cancer during her lifetime.[2] Conventional treatment for ovarian cancer consists of surgical reduction of tissue mass, usually followed by chemotherapy with platinum compounds combined with taxanes or oxazophosphorines.[3] Despite aggressive surgical intervention and intensive chemotherapy, the 5-year survival rates for patients with advanced ovarian cancer are less than 30%.[4] The clinical effectiveness of cisplatin-based combination therapy is commonly limited by intrinsic drug resistance, present at the outset or acquired during or after chemotherapy. Therefore, there is an urgent need for new cytotoxic drugs against ovarian cancer.

Among the metal complexes that exhibit striking similarities to cisplatin and exhibit antitumor activity are the early transition metal containing inorganic and organic complexes of vanadium(IV), among which *bis*(cyclopentadienyl)vanadium(IV) and peroxovanadates(V) were most thoroughly investigated.[5,6] The cytotoxic activity of cisplatin and vanadium compounds is believed to result mainly from their interaction with nuclear DNA. However, unlike cisplatin, which forms covalent DNA adducts which are potentially mutagenic,[7] vanadium complexes do not disrupt the Watson–Crick hydrogen bonding, but rather interact with nucleotide phosphate groups via a water bridge.[8] The antitumor *bis*(cyclopentadienyl) complexes of vanadium(IV) have a tetrahedral geometry with a 'bent sandwich' structure, whereas the oxovanadium(IV) complexes have a square pyramidal geometry with the oxoligand in the axial position.

In a systematic effort aimed at identifying new cytotoxic agents against ovarian cancer, we synthesized 15 oxovanadium compounds by modifying the ancillary ligands attached to the central vanadium(IV) atom as well as introducing substituents on these ligands and examined their cytotoxicity against four different human ovarian cancer cell lines. The peroxivanadates were stabilized with mono or biden-

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tate organic ligands such as phenanthroline, bipyridyl, bypyrimidyl or acetophenone, which form a five-membered ring with the vanadium(IV) atom. Structural variations of the ligands included addition of electron-donating (methyl) or electron-withdrawing (bromo, chloro and nitro) groups in positions 4, 5 and 7 on the phenanthroline, bipyridyl, bipyrimidyl or acetophenone rings. For the present study, the coordination complexes of oxovanadium(IV) [VO] were stabilized with: (i) five-membered *mono-* and *bis*-1,10-phenanthroline (phen); [VO(phen), VO(phen)$_2$, VO(Me$_2$-phen), VO(Me$_2$-phen)$_2$, VO(Cl-phen), VO(Cl-phen)$_2$, VO(NO$_2$-phen) and VO(NO$_2$-phen)$_2$]; (ii) *mono-* and *bis*-2,2′-bipyridyl (bipy); [VO(bipy), VO(bipy)$_2$, VO(Me$_2$-bipy) and VO(Me$_2$-bipy)$_2$]; (iii) *mono-* and *bis*-2,2′-bipyrimidyl (bipym); [VO(bipym) and VO(bipym)$_2$] and (iv) *bis*-5′-bromo-2′hydroxyacetophenone (acph) [VO(Br,OH-acph)$_2$], as ancillary ligands linked via nitrogen or oxygen atoms.

Our experimental results demonstrate that oxovanadium complexes of 1,10 phenanthroline, especially the *mono*-chelated, VO(NO$_2$-phen) and *bis*-chelated, VO(Me$_2$-phen)$_2$, VO(Cl-phen)$_2$ and VO(NO$_2$-phen)$_2$ complexes, were the most potent oxovanadium compounds and killed target cancer cells at low micromolar concentrations. The marked differences in the cytotoxic activity of oxovanadium(IV) complexes containing different heterocyclic ancillary ligands suggest that the cytotoxic activity of these compounds is determined by the identity of the five-member bidentate ligands, as well as the nature of the substituents on the heterocyclic aromatic rings. Our results presented herein provide experimental evidence that oxovanadium compounds induce apoptosis in human ovarian cancer cells. The lead compounds VO(Me$_2$-phen)$_2$ and VO(NO$_2$-phen)$_2$ may be useful in the treatment of ovarian cancer.

Materials and methods

Oxovanadium(IV) complexes containing 1,10-phenanthroline, 2,2′-bipyridyl, 2,2′-bipyrimidyl and acetophenone, and derivatives

The chemical structures of 15 oxovanadium(IV) compounds with either *mono-* or *bis*-1,10-phenanthroline, 2,2′-bipyridyl, 2,2′-bipyrimidyl and *bis*-acetophenone as ancillary ligands are depicted in Figure 1. Briefly, these complexes were synthesized by reacting an aqueous solution of vanadyl sulfate with an ethanol solution or a chloroform solution of the ligands (Figure 2). The complexes purified from chloroform, ether and/or water were characterized by Fourier transform infrared spectroscopy (FT-Nicolet model Protege 460; Nicolet Instrument, Madison, WI), UV-visible spectroscopy (DU 7400 spectrophotometer; Beckman Instruments, Fullerton, CA), mass spectrometry (Finnigan MAT 95 mass spectrometer; Madison, WI) and elemental analysis (Atlantic Microlab, Norcross, GA). These oxovanadium complexes have a square pyramidal geometry with the oxo ligand (O^{2-}) in the apical site. The peroxivanadates were stabilized with bidentate ligands which form a five-membered ring with the vanadium atom. Structural variations of the ligand included addition of bromo, chloro, methyl or nitro groups on the ancillary ligands in position 4, 7 or 5.

Cell lines and culture conditions

Four ovarian cancer cell lines were tested. SKOV-3 and ES-2 cells were propagated in McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS). PA-1 cells were cultured in Eagle's modified essential medium with 10% FBS, and OVCAR-3 cells were grown in RPMI 1640 with 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/l glucose, 1.5 g/l sodium bicarbonate, 0.01 mg/ml insulin and 20% FBS. All of the cell lines were cultured at 37°C in a humidified 5% CO_2 atmosphere.

Cell proliferation assay

To determine the growth-inhibitory effects of the oxovanadium compounds, we used the MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide]-based colorimetric assay for quantitation of cell proliferation and viability.[10] Briefly, exponentially growing ovarian cancer cells were seeded into a 96-well plate at a density of 2×10^4 cells/well and incubated in medium containing the oxovanadium(IV) compounds at concentrations ranging from 0.78 to 100 μM for 24 h at 37°C in a humidified 5% CO_2 atmosphere. Triplicate wells were used for each treatment. To each well, 10 μl of MTT (final concentration 0.5 mg/ml) was added and the plates were incubated at 37°C for 4 h to allow MTT to form formazan crystals by reacting with metabolically active cells. The formazan crystals were solubilized overnight at 37°C in a solution containing 10% SDS and 0.01 M HCl. The absorbance of each well was measured in a microplate reader (Labsystems) at 540 nm and a reference wavelength of 690 nm. To translate the A_{540} values into the number of live cells in each well, the A_{540} values were compared to those on standard A_{540} versus cell number curves generated for each cell line. The percentage of cell survival was calculated using the formula: % survival=[live cell number (test)/

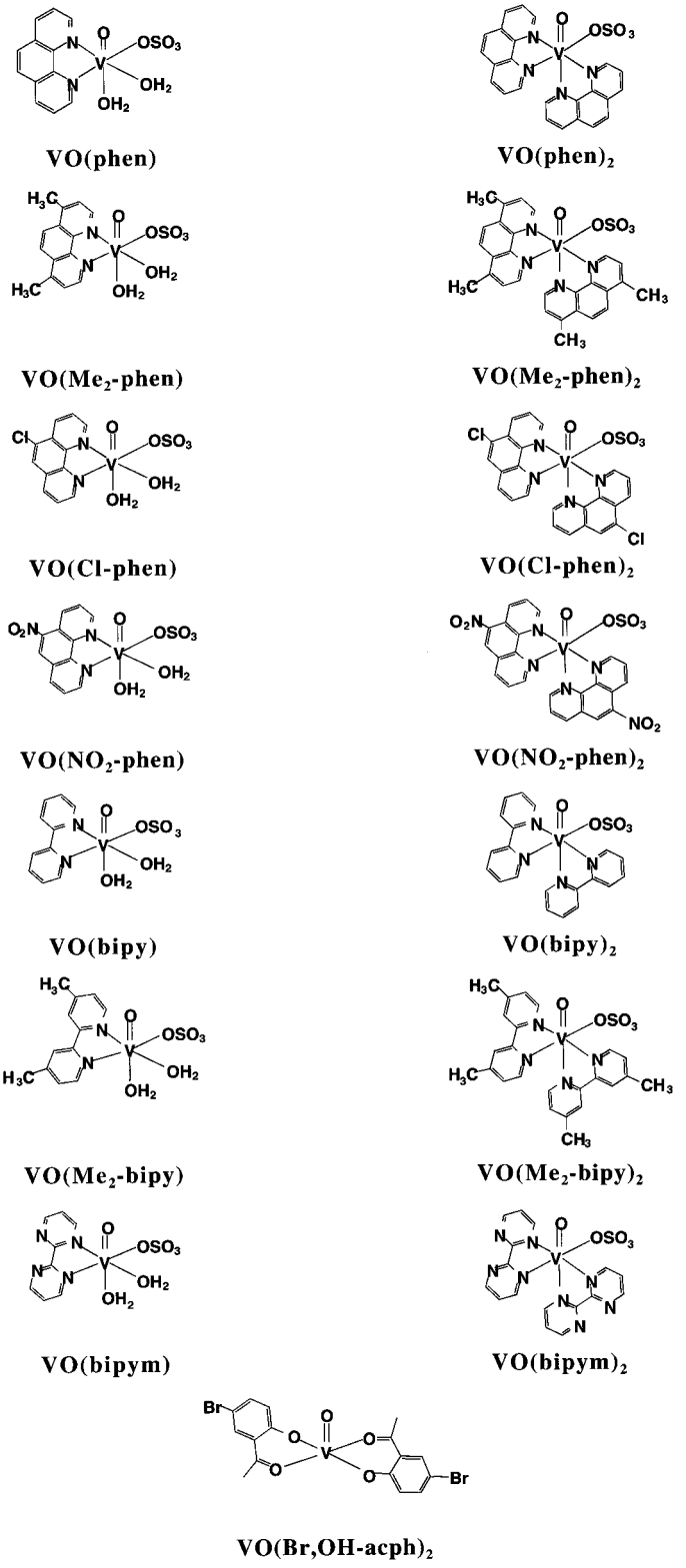

Figure 1. Chemical composition of 15 oxovanadium(IV) complexes. The oxovanadium(IV) complexes have a square pyramidal geometry with the oxo ligand (O^{2-}) in the axial position. These coordinated complexes are stabilized with five-membered *mono*- or *bis*-1,10 phenanthroline, 2,2′-bipyridyl, 2,2′-bypyrididyl and acetophenone-type bidentate ligands with the vanadium(IV) atom. Structural variations of the ligands were made after addition of bromo, chloro, methyl or nitro groups in positions 4, 5 or 7 on the ancillary ligands of the vanadium(IV) coordination sphere.

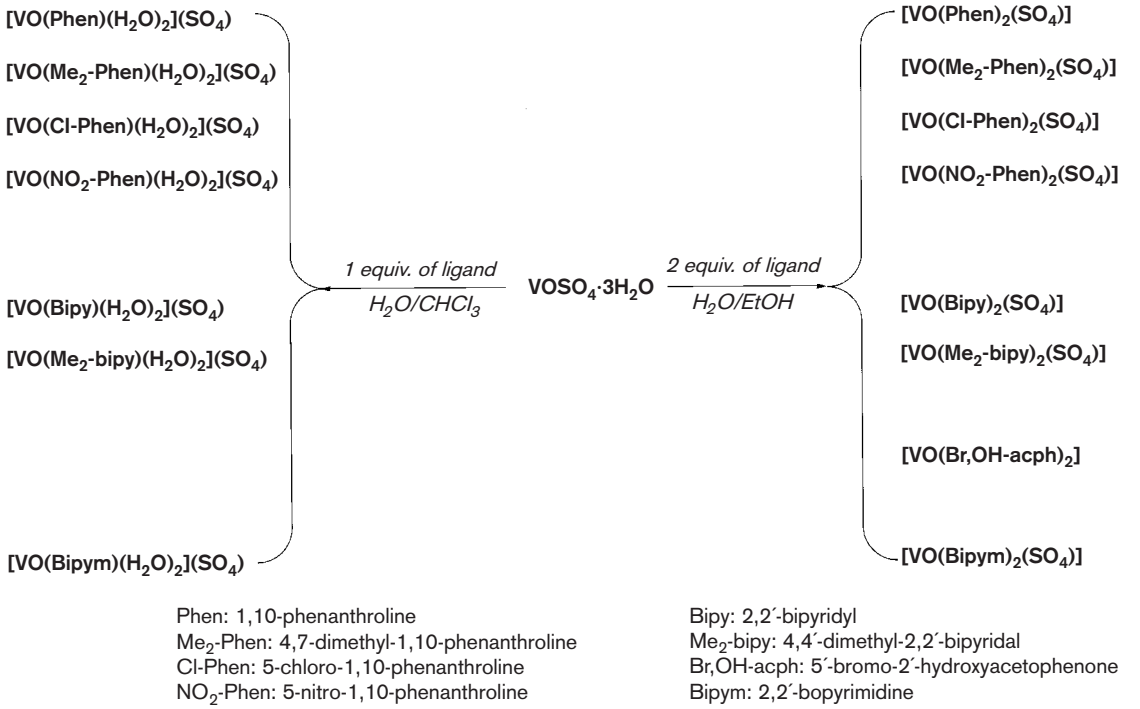

Figure 2. Scheme for the synthesis of 15 oxovanadium compounds.

live cell number (control)] × 100. The IC_{50} values were calculated by non-linear regression analysis using the Prism version 2.0 software (Graphpad Software, San Diego, CA).

Apoptosis assays

A flow cytometric two-color terminal dideoxynucleotidyl transferase (TdT)-mediated digoxigenin-uridine triphosphate (dUTP) nick-end-labeling assay (TUNEL) was employed to detect apoptotic nuclei.[11] Exponentially growing PA-1 and SKOV-3 cells (10^6/ml) were incubated in DMSO alone (0.05%) or treated with 50 μM each of the 15 oxovanadium compounds in 0.05% DMSO for 24 h. Cells were washed in PBS and fixed in 4% paraformaldehyde in PBS for 15 min on ice. After two washings in PBS, they were permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice and washed twice in PBS. Labeling of exposed 3′-hydroxyl (3′-OH) ends of fragmented nuclear DNA was performed using TdT and fluorescein isothiocyanate (FITC)-conjugated dUTP according to the manufacturer's recommendations (Boehringer Mannheim, Indianapolis, IN). Cells were counterstained with 5 μg/ml of propidium iodide (PI). Control samples included: (i) untreated cells and (ii) cells incubated with the reaction mixture without the TdT enzyme. Cells were analyzed with a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA). Relative DNA content (PI emission) was detected with band-pass filter 585/42 and dUTP incorporation (FITC emission) was detected with band-pass filter 530/30. Fluorescence was compensated for in the acquisition software using single-label control samples. Data was acquired in list mode, gated to 10 000 events per sample and analyzed using CellQuest software program (Becton Dickinson). Non-apoptotic cells do not incorporate significant amounts of dUTP due to lack of exposed 3′-OH ends and consequently have relatively little or no fluorescence compared to apoptotic cells which have an abundance of 3′-OH (M2 gates). Oxovanadium-induced apoptosis was detected by an increase in the number of cells staining with FITC–dUTP. The M1 and M2 gates were used to demarcate non-apoptotic and apoptotic PI-counterstained cell populations, respectively.

Confocal laser scanning microscopy (CLSM)

Morphological evidence for apoptosis was sought among the TUNEL-positive cells treated with and without 50 μM of $VO(phen)_2$, $VO(Cl\text{-}phen_2)$, $VO(Me_2\text{-}phen)_2$ and $VO(NO_2\text{-}phen)_2$ using CLSM. Confocal microscopy was performed using a BioRad MRC-1024 laser scanning confocal microscope (BioRad, Hercules,

CA) equipped with a krypton/argon mixed gas laser (excitation lines at 488, 568 and 647 nm) and mounted on a Nikon Eclipse E800 series upright microscope equipped with high numerical objectives. Using fluorescence imaging, the fluorescence emission of FITC and PI associated with PA-1 and SKOV-3 cells was simultaneously recorded using 598/40 nm and 680 DF32 emission filters, respectively. Confocal images were obtained using a Nikon ×60 (NA 1.4) objective and Kalman collection filter. Digitized data was processed using Lasersharp (BioRad) and digitized images were saved on a Jaz disk (Iomega, Roy, UT) and processed with Adobe Photoshop 5.5 software (Adobe Systems, Mountain View, CA). Final images were printed using a Fuji Pictrography 3000 (Fuji Photo Film, Tokyo, Japan) color printer.

DNA content/cell cycle distribution

The cell cycle distribution of cells was evaluated by flow cytometry. In brief, exponentially growing PA-1 cells were incubated with increasing concentrations (6.2, 12.5, 25 and 50 μM) of the oxovanadium compounds, VO(Me$_2$-phen)$_2$ and VO(NO$_2$-phen)$_2$, for 24 h at 37°C. Cells were harvested by trypsinization, washed in PBS, fixed with cold ethanol (70%) and stored at −20°C. For DNA analysis, they were centrifuged, suspended in 500 μl of PBS (10^6 cells/ml), treated with ribonuclease type IIA (100 μg/ml) for 30 min followed by the addition of 500 μl of PI (50 μg/ml in 0.1% Triton X-100 in 0.1% sodium citrate) for 30 min at room temperature and the DNA content was analyzed using a FACSCalibur flow cytometer (Becton Dickinson). The percentages of cells in G_0, G_1, S and G_2/M phases of the cell cycle were determined using CellQuest version 3.1. Results were expressed as the percentage of cells in sub-diploid ($<2N$), G_0+G_1, S and G_2+M phases of the cell cycle.

Results

Cytotoxicity of oxovanadium(IV) compounds against human ovarian cancer cells

Among the 15 oxovanadium(IV) compounds evaluated, each of the eight oxovanadium complexes of *mono-* or *bis*-chelated ligands of 1,10-phenanthroline exhibited significant cytotoxicity against the ovarian cancer cell lines at low micromolar concentrations (Table 1). Oxovanadium(IV) complexes with *mono-* or *bis*-chelated ligands of bipyridyl, bipyrimidyl and acetophenone were far less active. The cytotoxic activity of phenanthroline-linked oxovanadium(IV) complexes was strongly dependent on the type of coordinated heteroligands. When compared with diaqua *mono*-chelated complexes, the octahedral

Table 1. Cytotoxic activity of 15 oxovanadium(IV) compounds against human ovarian cancer cell lines

Treatment	IC_{50} (μM)[a]				Percent apoptosis at 50 μM[b]	
	PA-1	SKOV-3	ES-2	OVCAR-3	PA-1	SKOV-3
1,10-phenanthroline-linked compounds						
VO(phen)	35.5	27.4	93.7	56.7	52.6±4.0[c]	27.6±15.5
VO(phen)$_2$	42.0	63.9	82.2	50.3	66.2±15.5	16.6±3.7
VO(Me$_2$-phen)	22.6	84.5	>100	53.6	39.2±3.0	ND
VO(Me$_2$-phen)$_2$	8.4	6.5	20.6	43.9	97.6±1.5	26.5±4.9
VO(Cl-phen)	13.9	63.9	49.7	81.4	52.6±2.5	25.3±12.0
VO(Cl-phen)$_2$	8.4	15.3	17.1	54.7	52.5±11.1	62.6±5.8
VO(NO$_2$-phen)	14.5	14.6	15.4	70.8	52.3±1.5	75.3±5.8
VO(NO$_2$-phen)$_2$	22.5	24.9	18.3	28.5	72.3±2.0	73.0±10.5
Bipyridyl-linked compounds						
VO(bipy)	>100	>100	>100	83.7	2.6±0.5	8.0±4.3
VO(bipy)$_2$	54.5	>100	>100	84.6	28.0±2.6	3.2±2.3
VO(Me$_2$-bipy)	<100	82.3	>100	>100	2.5±0.4	5.4±4.8
VO(Me$_2$-bipy)$_2$	18.2	>100	>100	>100	29.2±7.5	10.3±5.5
Bipyrimidyl-linked compounds						
VO(bipym)	81.6	>100	>100	>100	1.8±0.2	1.6±0.4
VO(bipym)$_2$	75.2	>100	>100	>100	1.8±0.3	2.7±0.1
Acetophenone-linked compounds						
VO(Br, OH-acph)$_2$	>100	>100	>100	>100	2.3±0.5	3.7±2.2

[a]PA-1, SKOV-3, ES-2 and OVCAR-3 cells were treated with increasing 2-fold concentrations (0.78–100 μM) of 15 oxovanadium(IV) complexes for 24 h; cell viability was measured using MTT assays and IC_{50} values were calculated by non-linear regression analysis.
[b]TUNEL-positive cells were quantitated by the two-color flow cytometric assay.
[c]Data are mean±SD of three independent experiments. ND, not determined.

structure oxovanadium(IV) compounds stabilized with five-member *bis*-chelated ligands of 1,10-phenanthroline showed superior cytotoxic activity against all four ovarian cancer cell lines. The *mono*-chelated [VO(NO_2-phen)], *bis*-chelated dimethyl-phen [VO(Me_2-phen)$_2$ and *bis*-chelated chloro-phen [VO(Cl-phen)$_2$] complexes were the most potent oxovanadium(IV) compounds with IC_{50}s ranging from 14.5 to 70.8, 6.5 to 43.9 and 8.4 to 54.7 μM, respectively (Table 1). Notably, The *bis*-chelated dinitro-phen compound, VO(NO_2-phen), was cytotoxic against all four ovarian cancer cell lines tested with IC_{50}s values ranging from 18.3 to 28.5 μM. Thus, the presence of phenanthroline rings as well as dimethyl, dinitro or chloro substitutions enhance the potency of anticancer activity because unsubstituted *mono*- and *bis*-chelated oxovanadium(IV) complexes of 1,10-phenanthroline [VO(phen) or VO(phen)$_2$] were less active (IC_{50}s ranging from 27.4 to 93.7 and 42 to 82.2 μM, respectively).

Overall, PA-1, SKOV-3 and ES-2 cells were equally sensitive to VO(Me_2-phen)$_2$, VO(NO_2-phen)$_2$ and VO(Cl-phen)$_2$. The cisplatin-resistant cell line, OVCAR-3, was highly sensitive to VO(NO_2-phen)$_2$. Figure 3 shows the concentration-dependent MTT-based cytotoxicity curves of four representative oxovanadium(IV) complexes of 1,10-phenanthroline against PA-1, SKOV-3, ES-2 and OVCAR-3 cells, respectively. The marked differences in the cytotoxic activity of oxovanadium(IV) compounds containing different heterocyclic ancillary ligands suggest that the cytotoxic activity of these compounds is determined by the identity of the five-member bidentate ligands, as well as by the nature of the substituents on the heterocyclic aromatic rings.

Oxovanadium(IV) complexes of 1,10-phenanthroline induce apoptosis in human ovarian cancer cells

To determine whether the cytotoxicity of the oxovanadium(IV) compounds is associated with apoptotic cell death, PA-1 and SKOV-3 ovarian cancer cell lines were cultured with each of the 15 oxovanadium(IV) compounds (50 μM) for 24 h and then subjected to flow cytometric analysis of dUTP incorporation by the TdT-mediated TUNEL assay. Among the 15 oxovanadium complexes evaluated by the flow cytometric TUNEL assay, only the oxovana-

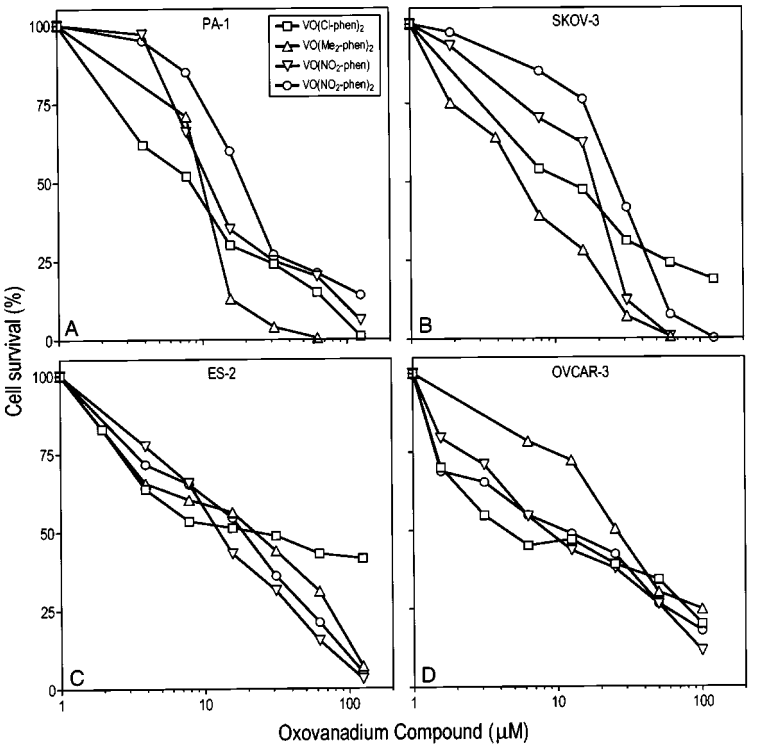

Figure 3. Cytotoxicity of oxovanadium compounds with *bis*-phen ligand and methyl, nitro or chloro substitutions against human ovarian cancer cell lines, PA-1, SKOV-3, ES-2 and OVCAR-3. Cells were incubated with increasing concentrations (0.78–100 μM) of the compounds, VO(Me_2-phen)$_2$, VO(NO_2-phen)$_2$ and VO(Cl-phen)$_2$ for 24 h in 96-well plates, and the cell proliferation/viability was measured using MTT assays. Each point represents the mean of triplicate values.

dium(IV) complexes of 1,10 phenanthroline caused a marked increase in TUNEL-positive nuclei ranging from 39 to 97% for PA-1 cells and 17 to 75% for SKOV-3 cells, respectively (Table 1). Figure 4(A, C and E) depicts the two-color (FITC and PI) flow cytometric contour plots of cells from representative TUNEL assays. Control PA-1 cells were treated for 24 h at 37°C with 0.05% DMSO, whereas test cells were treated for 24 h at 37°C with two cytotoxic oxovanadium(IV) complexes, $VO(Me_2\text{-phen})_2$ and $VO(NO_2\text{-phen})_2$, at a final concentration of 50 μM. The TdT-dependent incorporation of FITC-dUTP was dramatically increased in cells treated with cytotoxic oxovanadium(IV) compounds as a result of abundance of free 3′-hydroxyl DNA ends created by apoptotic DNA fragmentation (Figure 4B, D and F). Cells treated with $VO(Me_2\text{-phen})_2$ and $VO(NO_2\text{-phen})_2$ showed an abundance of apoptotic nuclei at 24 h after treatment. The majority of oxovanadium(IV) compound-treated cells displayed the characteristic morphological features of apoptotic cell death, including marked shrinkage, chromatin condensation, nuclear fragmentation, appearance of typical apoptotic bodies and inability of the cells to adhere to the substratum. Apoptosis after treatment with two representative oxovanadium(IV)

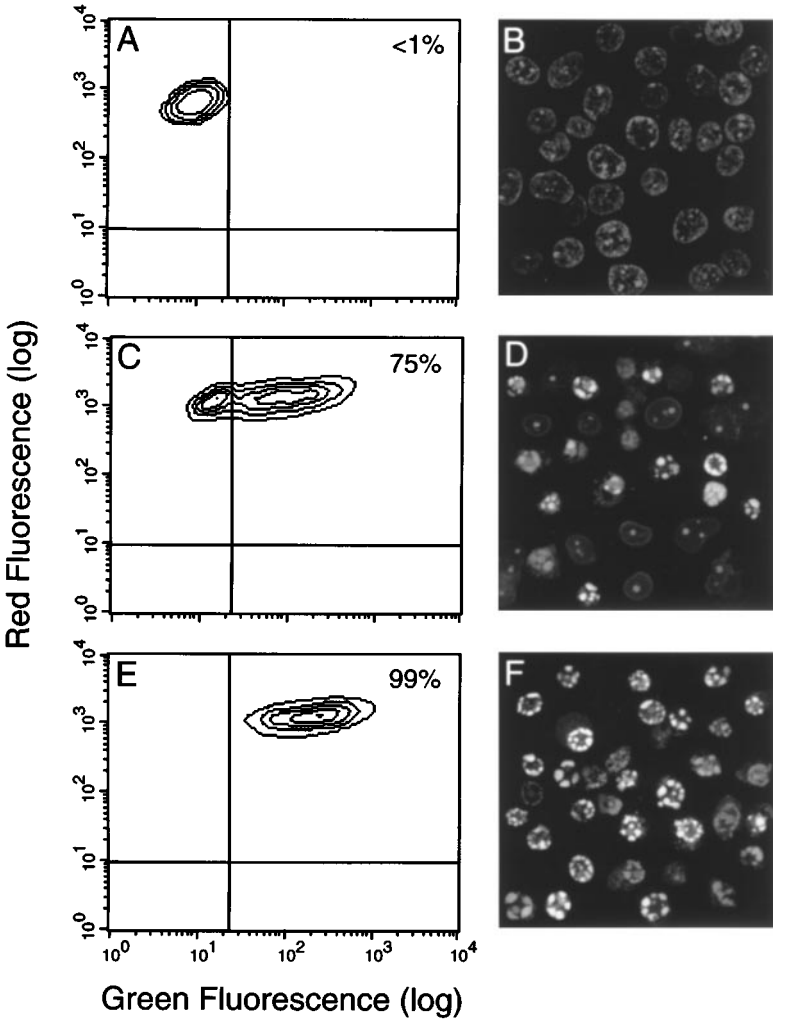

Figure 4. Oxovanadium(IV) compounds induce apoptosis in human ovarian cancer cells. PA-1 cells were incubated with oxovanadium compounds $VO(Me_2\text{-phen})_2$ and $VO(NO_2\text{-phen})_2$ (50 μM) for 24 h, fixed, permeabilized and visualized for DNA fragmentation in a TUNEL assay using TdT and FITC–dUTP. Cell nuclei were counterstained with PI (red color). Two-color flow cytometric contour plots (left panels; A, C and E) and confocal laser scanning microscopy images (right panels; B, D and F) of apoptotic nuclei of PA-I cells treated with $VO(Me_2\text{-phen})_2$ (C and D) and $VO(NO_2\text{-phen})_2$ (E and F) or vehicle (A and B). Percentages in the *left panels* indicate cells with apoptotic nuclei.

compounds, VO $(Me_2\text{-phen})_2$ and VO$(NO_2\text{-phen})_2$, was also evident from the concentration-dependent emergence of a hypodiploid ($<2N$) peak in the DNA histograms of PI-stained nuclei (Figure 5). Similar data was obtained with SKOV-3 cells (not shown).

Discussion

To our knowledge, this is the first report on structure–activity relationships affecting the anticancer activity of oxovanadium(IV) complexes against human ovarian cancer cell lines. Our studies demonstrated that: (i) the phenanthroline-containing oxovanadium(IV) complexes were the most potent cytotoxic agents, with bidentate complexes being more active than the monodentate complexes; (ii) the substitution of electron-withdrawing chloro or nitro groups as well as an electron-donating methyl group on the phenanthroline rings at the 4, 5 or 7 position further enhanced the potency of anticancer activity of these oxovanadium(IV) complexes and (iii) the cytotoxic activity of oxovanadium(IV) complexes was associated with apoptotic cell death in ovarian cancer cells. Such oxovanadium(IV) compounds are best represented by VO$(Me_2\text{-phen})_2$, VO$(NO_2\text{-phen})_2$ and VO(Cl-phen)$_2$ in our study.

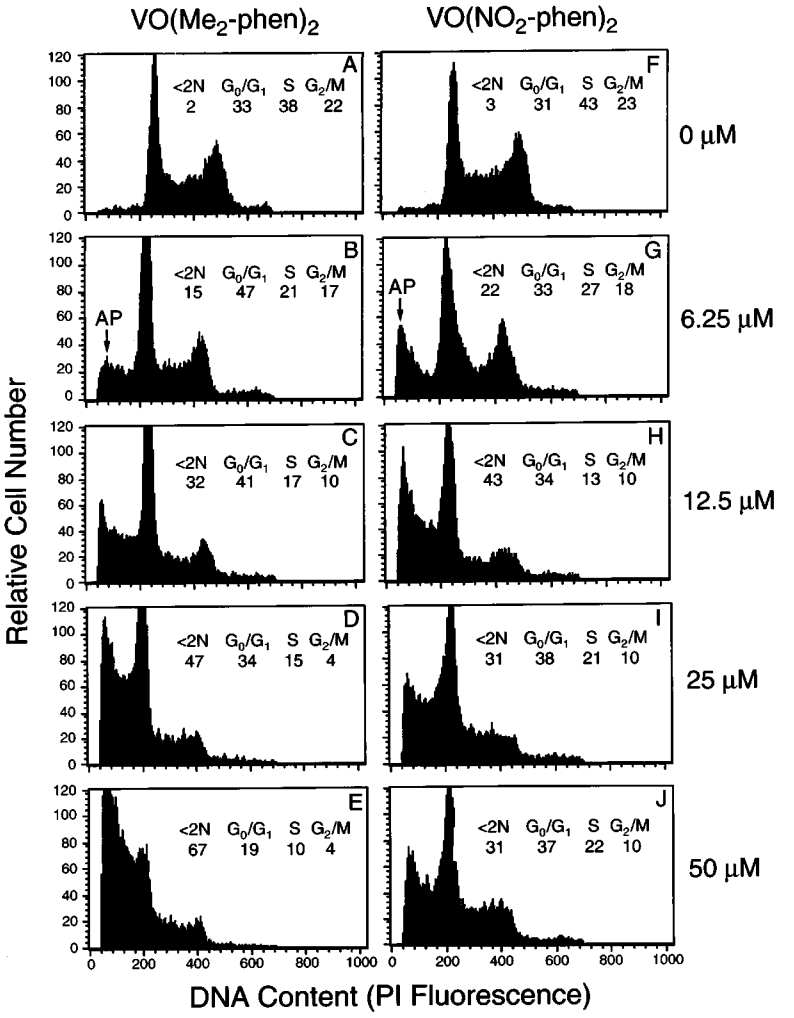

Figure 5. Oxovanadium complexes affect DNA content/cell cycle distribution in human ovarian cancer cells. PA-1 cells were treated with vehicle or 6.25, 12.5, 25 or 50 μM of oxovanadium compounds, VO$(Me_2\text{-phen})_2$ and VO$(NO_2\text{-phen})_2$, for 24 h at 37°C, trypsinized, fixed, stained with PI and analyzed by flow cytometry for DNA content. The percentages of cells in each cell cycle were determined using CellQuest software and are indicated in each of the histograms. An increase in G_2/M cells and a selective loss of $G_{0/1}$ cells preceeded apoptosis. The presence of apoptotic nuclei was evident from the hypodiploid sub-G_1 peak (AP).

Several organic and inorganic complexes of vanadium have been tested for *in vitro* as well as *in vivo* antitumor activity, among which the *bis*(cyclopentadienyl)vanadium(IV) or vanadocenes and peroxovanadates(V) were found to be most promising alternatives to cisplatin.[5,6,12–19] It was proposed that DNA is the target for vanadocene complexes.[6,19] Peroxovanadates(V) were proposed to undergo one-electron intramolecular transfer, producing vanadium(IV) and superoxide, a process that triggers the generation of other reactive oxygen species (ROS), including hydroxyl radical, which ultimately causes cell death.[20] Peroxovanadates(V) were also shown to inhibit protein tyrosine phosphatases, the function of which is essential for mitosis progression, thereby inhibiting the cell cycle.[20] In our study all eight oxovanadium(IV) complexes of 1,10-phenanthroline showed substantial *in vitro* anticancer activity and induced apoptotic cell death, with $VO(Me_2\text{-phen})_2$ being the most potent.

In a previous study, these oxovanadium(IV) compounds were shown to have potent spermicidal and apoptosis-inducing property against human sperm at low micromolar concentrations.[21] This spermicidal activity was proposed to be mediated by the reactive oxygen intermediates inducing activity of oxovanadium(IV) compounds. Although we do not know the molecular basis for the *in vitro* anticancer properties of our oxovanadium(IV) complexes at the present time, it has been shown that this type of compounds can interact with DNA and causes DNA cleavage.[22] Our previous studies and others suggested that the generation of ROS and the cell cycle arrest by the compounds may also contribute to the antitumor activity.[9,23] The presence of two phenanthroline rings and dimethyl, dinitro or chloro substitution on phenanthroline ligands of oxovanadium(IV) complexes substantially improved the cytotoxic activity. This superior activity may be due to the electron-donating dimethyl or electron-withdrawing chloro and dinitro groups, which may contribute to the generation of ROS,[24,25] to the cell permeable nature of the complex coupled with the intrinsic metal chelating activity,[26] and to p53 transactivation activity of phenanthroline. The transactivation of p53 by phenanthroline was shown to induce p53 target genes such as Waf-1 and Mdm-2;[27] this induction results in cell cycle arrest at G_1 and apoptosis.[28]

In conclusion, our results provide experimental evidence that oxovanadium(IV) complexes of 1,10-phenanthroline and derivatives, linked to vanadium (IV) via nitrogen atom have potent *in vitro* anticancer activity against human ovarian cancer cells. The potential therapeutic applications of vanadium complexes *in vivo*, particularly to suppress tumor cell growth via apoptosis with relatively few adverse effects has sparked interest as a new class of anticancer agents.[12,16,17,29] Because of its potent apoptosis-inducing activity, further development of our lead compound $VO(Me_2\text{-phen})_2$ may provide the basis for the design of effective treatment programs for ovarian cancer patients.

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