Bioorganic & Medicinal Chemistry Letters 18 (2008) 6494–6500

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Five-coordinated oxovanadium(IV) complexes derived from amino acids and ciprofloxacin: Synthesis, spectral, antimicrobial, and DNA interaction approach

M. N. Patel *, S. H. Patel, M. R. Chhasatia, P. A. Parmar

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar, 388 120, Gujarat, India

ARTICLE INFO

Article history:
Received 15 May 2008
Revised 9 September 2008
Accepted 13 October 2008
Available online 21 October 2008

Keywords:
Oxovanadium
DNA intercalation
Ciprofloxacin
Gel electrophoresis
Viscometric studies
SOD-like activity

ABSTRACT

Five-coordinated oxovanadium(IV) complexes with ciprofloxacin and various uninegative bidentate amino acids have been prepared. The structure of complexes has been investigated using spectral, physicochemical, mass spectroscopy, and elemental analyses. The antimicrobial activities (MIC) of the complexes, ligands, metal salt, and some standard drugs have been evaluated using the doubling dilution technique against *Staphylococcus aureus, Baccilus subtilis*, *Serratia marcescens* (*Gram-positive*), and *Pseudomonas aeruginosa*, and *Escherichia coli* (Gram-negative) bacteria. The result shows the significant increase in the antibacterial activity of the ligand, metal, and ciprofloxacin on complexation. The interaction of the complexes with pBR322 DNA has been investigated using spectroscopic, gel electrophoresis, and viscometric techniques. This shows that the complexes can bind to pBR322 DNA by the intercalative mode. The superoxide dismutase-like activity of the complexes has been determined.

The development of metal complexes as artificial nucleases is an area of burgeoning interest. The metal complexes as pharmaceuticals[1] have gained access over traditional organic dominated drugs, due to their potential use as regulators of gene expression and tools of molecular biology.[2] The major intracellular target of anticancer metallodrugs is DNA; therefore metal complexes that can bind to specific nucleotides of DNA are of interest. Studies of metal complexes, which react at specific sites along a DNA strand as reactive models for protein–nucleic acid interactions, provide routes toward the rational development of chemotherapeutic agents,[3] sensitive chemical probes for DNA structure in solution,[4] and tools for the molecular biologist to dissect genetic systems.[5] In this regard, transition metal complexes are outstanding as an artificial nuclease for DNA due to their diverse ability to recognize and react selectively with individual target sites.[6] Moreover, ciprofloxacin a quinolone-type compound is widely used as an anti-bacterial drug that targets the DNA topoisomerase (gyrase) of bacterial type II. Treatment with this drug leads to the breaking up of DNA double-strand leading to cell death. The cytotoxicity of the drug is achieved via strong binding of the complex to the gyrase–DNA in the presence of metal. At the same time, its therapeutic mode does not have a purely organic path; some are activated by biotransformation, while others have a direct or indirect effect of metal ion on its metabolism.[7–9] To continue research in DNA-binding model of the fluoroquinolones and their transition metal complexes,[10] in this letter, we have prepared the VO(IV) mixed-ligand complexes of ciprofloxacin and DL-alanine (L^1) or L-tyrosine (L^2) or L-tryptophan (L^3) or glutamic acid (L^4) or L-leucine (L^5). The DNA-binding properties of the complexes have been investigated by ultraviolet spectroscopy, viscosity measurements, and gel electrophoresis. Experimental results indicated that the complexes and ciprofloxacin can bind to DNA by intercalation modes, but the binding affinity of the complexes is much higher than that of the ligand.

All the chemicals used were of analytical grade. Vanadylsulphate, DL-alanine, L-tyrosine, L-tryptophan, glutamic acid and L-leucine were purchased from, E. Merck (India) Ltd., Mumbai. Ciprofloxacin hydrochloride was purchased from Bayer AG (Wuppertal, Germany). Luria broth, ethidium bromide, sucrose, and tris(hydroxymethyl)methylamine were purchased from Hi-media Laboratories Pvt., Ltd., India. Agarose was purchased from Sisco Research Lab., India. Bromophenol blue, acetic acid, and EDTA were purchased from SD Fine Chemicals, India. The organic solvents were purified by standard methods.[11]

Perkin-Elmer elemental analyzer (240) was used to analyze carbon, hydrogen, and nitrogen. Thermogravimetric analysis and differential scanning calorimetric study were performed with a model 5000/2960 SDTA, TA instrument (USA). Infrared spectra were recorded on an FT-IR Shimadzu spectrophotometer as KBr pellets in the range 4000 to 400 cm^{-1}. The electronic spectra of the complexes were recorded in the range 800 to 200 nm on UV-160A UV–vis spectrophotometer, Shimadzu, Japan. The magnetic moments were measured by Gouy's method using mercury tetrathiocyanatocobaltate(II) as the calibrant ($\chi_g = 16.44 \times 10^{-6}$ cgs

* Corresponding author.
E-mail addresses: jeenenpatel@yahoo.co.in, jeenen@gmail.com (M.N. Patel).

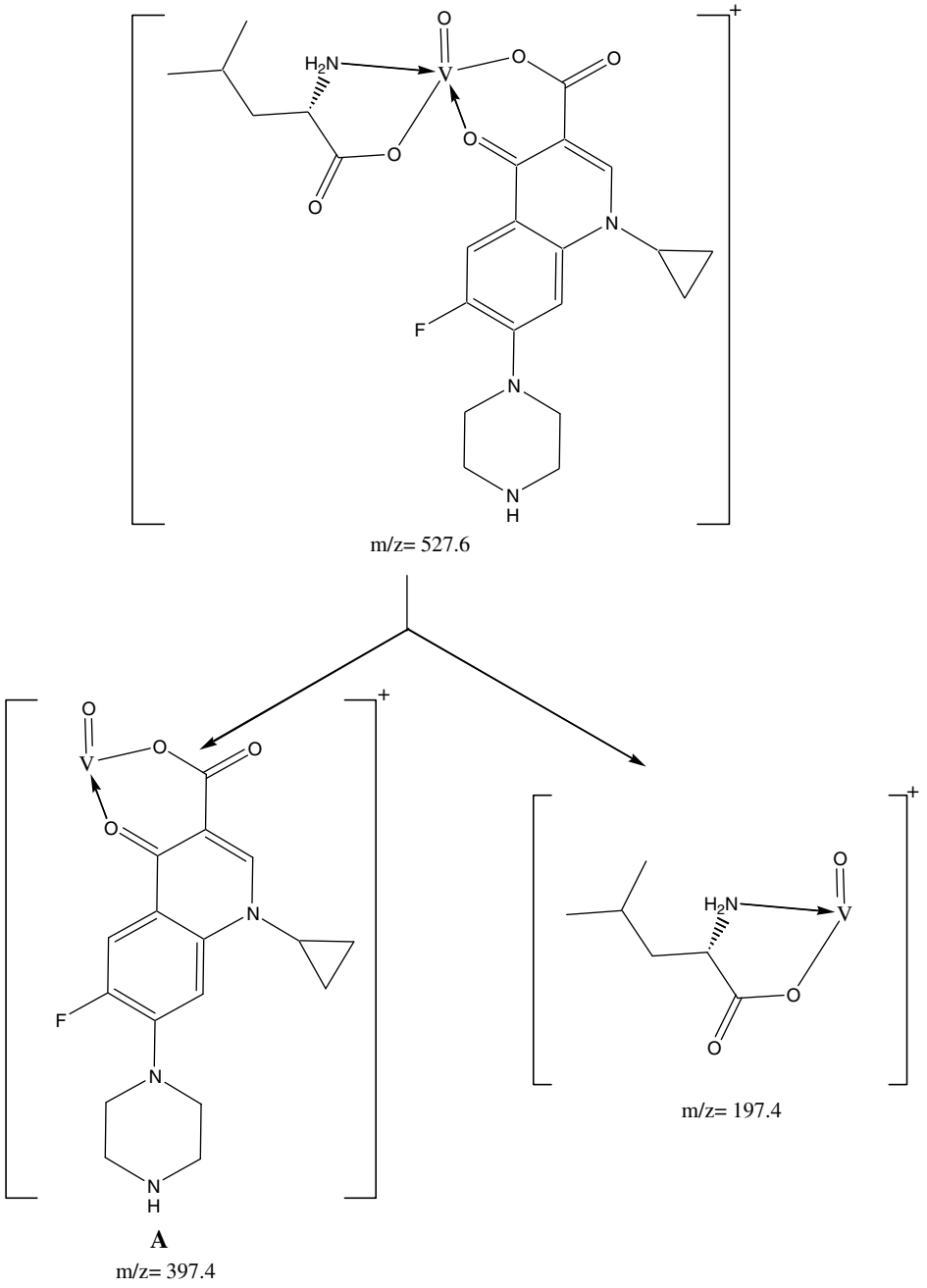
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.10.068

Table 1
Infrared spectral data of the complexes(cm^{-1})

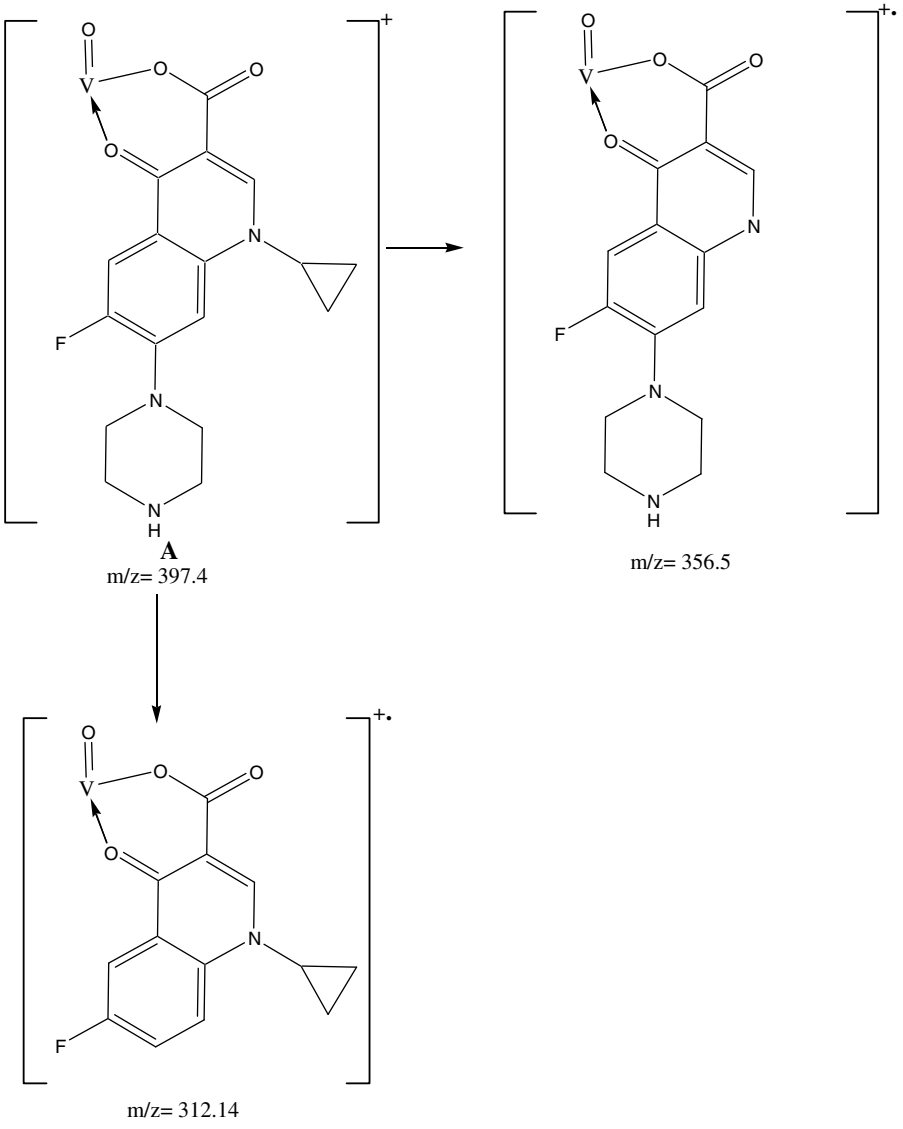
Complexes	ν(C=O) pyridone	$\nu(COO)_{sy}$	$\nu(COO)_{as}$	$\Delta\nu$	$\nu(M-O)_{Carbo}$	ν(M–N)	ν(C–F)	ν(C–N)	ν(V=O)
I	1619	1375	1587	212	426	530	1276	1356	1001
II	1620	1371	1586	215	427	525	1275	1357	1020
III	1635	1370	1589	219	428	531	1277	1356	1021
IV	1625	1373	1587	214	427	534	1278	1354	1020
V	1677	1371	1585	214	426	535	1274	1357	1020

units at 20 °C), Citizen Balance. The diamagnetic correction was made using Pascal's constant.[12] Mass spectra were recorded on Shimadzu LCMS 2010.

All the synthesized complexes are stable to air for an extended period of time and soluble in DMSO, slightly soluble in ethanol and water; insoluble in benzene, acetone, acetonitrile, and diethyl ether. Elemental analyses of the complexes are in good agreement with theoretical expectation. They possess high melting points indicating that the complexes are stable in air.



Scheme 1. Proposed fragmentation pattern of $[C_{23}H_{33}FN_4O_8V]$.



Scheme 1. (*continued*)

Table 2
Electronic absorption parameters of VO(IV) complexes (nm)

Complexes	λ_4	λ_3	λ_2	λ_1	$\Delta(\lambda_1 - \lambda_2)$
I	405	548	606	825	219
II	404	530	606	829	223
III	399	545	580	825	245
IV	401	530	591	829	238
V	405	547	604	790	186

The absorption bands observed at 1624 and 1340 cm^{-1} in ciprofloxacin are assigned to be $\nu(COO)_{asy}$ and $\nu(COO)_{sym}$, respectively, while in mixed-ligand complexes these bands observed at 1585–1589 and 1370–1375 cm^{-1}. The frequency separation ($\Delta\nu = \nu(COO)_{asy} - \nu(COO)_{sym}$) in investigated mixed-ligand complexes is greater than 200 cm^{-1}, suggests that the carboxylato group posses unidentate nature[13] and also the ν(C=O) stretching vibration band appearing at 1708 cm^{-1} in the spectra of ciprofloxacin, while in complexes this band is shifted toward lower energy at 1619–1677 cm^{-1}, which suggests that coordination occurs through pyridone oxygen atom.[14] The absorption bands observed at 525–535 cm^{-1} are attributed to ν(M–N). The sharp band in ciprofloxacin at 3520 cm^{-1} is due to hydrogen bonding; [15], which is attributed to ionic resonance structure and peak observed because of the stretching vibration of free hydroxyl group. This band completely vanished in the spectra of mixed-ligand complexes indicates deprotonation of carboxylate proton. Of particular interest are the spectroscopic features of the conformers in the 1100 to 850 cm^{-1} region, which after screening the bands due to ligand internal stretching, reveal interesting differences in their metal–terminal oxygen (V=O_t) vibrational modes. The metal–terminal oxygen (V=O_t) band observed at ~1000 cm^{-1} region suggesting the anti conformers and vanadium has a distorted square-pyramidal structure.[16] The complexes have one unpaired electron, characteristic of vanadyl unit, and a fairly high V=O stretching frequency in the infra-red spectrum about 1000 cm^{-1}, suggesting that there is no ligand (or a weakly bound solvent) in the sixth position.[17] Some prominent IR band frequencies of the compounds are provided in Table 1.

The mass spectra of the complex (V) ($C_{23}H_{33}FN_4O_8V$) reveals that the peak at m/z = 527.6 stands for the molecular ion peak of

Table 3
Thermal analysis data of the complexes

Complexes	TG range (°C)	Assignment
I	50–130	Loss of two lattice water molecule
	140–410	Removal of the ligand
	410–700	Leaving V_2O_5 as residue
II	50–120	Loss of two lattice water molecule
	130–420	Removal of the ligand
	420–710	Leaving V_2O_5 as residue
III	50–120	Loss of two lattice water molecule
	120–400	Removal of the ligand
	410–720	Leaving V_2O_5 as residue
IV	50–130	Loss of two lattice water molecule
	140–410	Removal of the ligand
	410–690	Leaving V_2O_5 as residue
V	50–130	Loss of two lattice water molecule
	130–400	Removal of the ligand
	410–700	Leaving V_2O_5 as residue

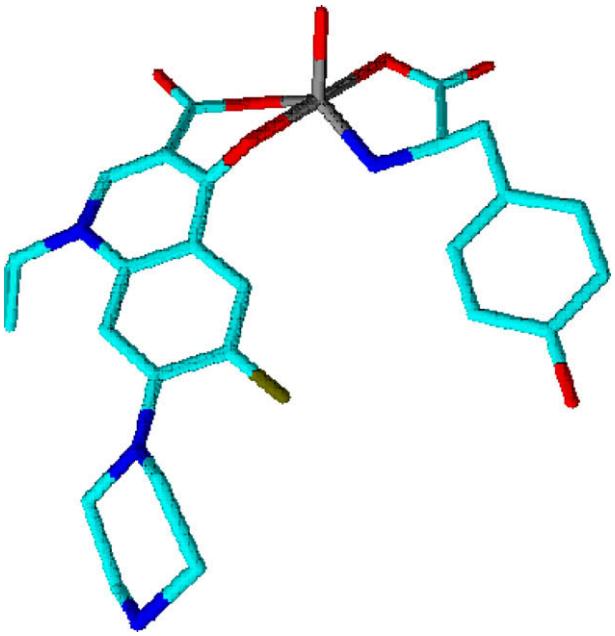


Figure 1. Probable structure of the complex (II).

Table 4
MIC data of the compounds (μM)

Compound	Gram positive			Gram negative	
	S. aureus	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
$VOSO_4{\cdot}3H_2O$	2568.35	2568.35	2568.35	1580.52	1185.39
Norfloxacin	2.50	2.50	4.07	3.75	2.81
Ofloxacin	1.93	1.38	1.66	2.21	1.38
Pefloxacin	2.09	2.39	5.09	5.69	2.69
Sparfloxacin	1.27	2.03	1.52	1.52	1.27
Levofloxacin	1.66	2.21	1.66	1.66	0.96
Enrofloxacin	1.94	3.89	1.66	1.39	1.39
Gatifloxacin	5.06	3.99	2.93	1.01	2.93
Ciprofloxacin	1.63	1.08	1.63	1.36	1.36
I	0.57	0.76	0.76	0.95	2.87
II	1.63	1.63	1.63	1.63	3.26
III	0.62	0.78	0.78	3.92	3.92
IV	1.72	1.72	0.86	4.31	4.31
V	1.77	0.88	0.88	4.43	1.77

complex (without water of crystallization) where as several peaks observed at 397.4, 197.4, 312.14, and 356.5 *m*/*z* value corresponds to the fragments as shown in scheme 1.

The UV–vis spectra of the complexes have been recorded using UV-160A UV–vis spectrophotometer, Shimadzu, Japan. The four absorption bands observed in mixed-ligand complexes at ~810 nm for $d_{xy} \rightarrow d_{xz}$ (band I), ~590 nm for $d_{xy} \rightarrow d_{yz}$ (band II), at ~540 nm for $d_{xy} \rightarrow d_{x^2-y^2}$ (band III), and ~405 nm for $d_{xy} \rightarrow d_z^2$ (band IV). An examination of Table 2 substantiates that the parameter, Δ ($\lambda_1 - \lambda_2$), related to the splitting of the d_{yz} and d_{xz} levels, can be used for establishment of the geometrical distortion. Increasing order for Δ ($\lambda_1 - \lambda_2$), for the synthesized complexes is V (186 nm) < I (219 nm) < II (223 nm) < IV (238 nm) < III (245 nm).The mixed-ligand complexes exhibit magnetic moment of 1.68–1.73 BM. This value is close to the spin-only value expected for $s = 1/2$ system (1.73 BM), which may be indicative of distorted square-pyramidal geometry around the VO(IV) ion.

The thermogravimetric analyses for the mixed-ligand complexes were carried out with a temperature ranging from 20 to 800 °C in N_2 atmosphere at a rate of 10 °C per minute in order to establish their compositional differences as well as to ascertain the nature of associated water molecules.[18] The determined temperature ranges and corresponding percent mass loss accompanying the changes in the mixed-ligand complexes on heating revealed the following things. The TG curves of mixed-ligand complexes show three-decomposition steps. It has been observed that all the mixed-ligand complexes shows a loss in weight corresponding to two water molecules in the range of 50–130 °C which indicating that these water molecules are water of crystallization. In second step weight loss during 130–420 °C is corresponding to liberation of ligand and leaving behind the oxide of metal in the temperature range 420–710 °C. Thermal analysis data for complexes are given in the Table 3. Suggested structure of the complexes from the above analytical facts is given below in Figure 1.

The complexes exhibit strong activities against two Gram(−ve), i.e., *Escherichia coli* and *Pseudomonas aeruginosa*, and three Gram(+ve), i.e., *Staphylococcus aureus, Baccilus subtilis, Serratia marcescens* microorganisms. The results concerning in vitro antimicrobial activity (MIC) of the ligands, complexes, metal salt, and standard drugs are represented in Table 4. The ligands (L^1–L^5) exhibit no antimicrobial activity. Here, in case of *S. aureus* the complexes I (MIC = 0.57 μM) and III (MIC = 0.62 μM) are more potent than the standard drugs, whereas complex II and ciprofloxacin are equipotent (MIC = 1.63 μM). In the case of *B. subtilis* complexes I (MIC = 0.76 μM), III (MIC = 0.78 μM), and V (MIC = 0.88 μM) prove to be more potent compared to the tested standard drugs which stood along with ciprofloxacin, whereas the remaining two are less potent compare to ciprofloxacin and ofloxacin. In the case of *S. marcescens* complexes I (MIC = 0.76 μM), III (MIC = 0.78 μM), IV (MIC = 0.86 μM), and V (MIC = 0.88 μM) are active compared to the standard drugs used herein out of which complex I is the most potent of all. For *P. aeruginosa* except complex I (MIC = 0.95 μM) all other complexes are less active than the standard drug. But in the case of *E. coli*; none of the synthesized compounds proved to be active but still complex V (MIC = 1.77 μM) leads among all the five. No doubt all the complexes exhibit much higher antimicrobial activity than the metal salt and ligands but complex I proven to be the most active. It was observed that all the complexes were proven to be more potent bacteriostatic compared to ligands. The inhibition activity seems to be governed in certain degree by the facility of coordination at the metal center. This may support the argument that some type of bimolecular binding to the metal ions or intercalation or electrostatic interactions causing the inhibition of biological synthesis and preventing the organisms from reproducing. The strong antimicrobial activities of these compounds

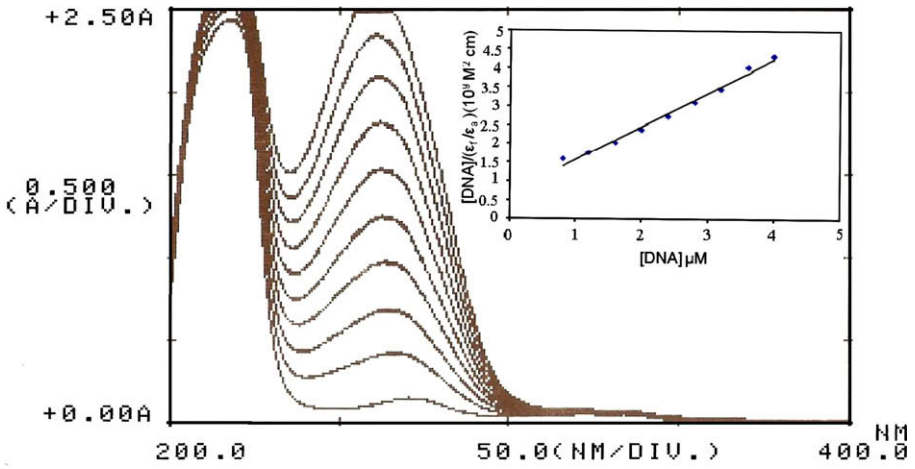


Figure 2. Absorption titration spectra of complex (II).

Table 5
The binding constants (K_b) of VO(IV) complexes with DNA in phosphate buffer pH 7.2

Complexes	K_b (M^{-1})
I	7.5×10^4
II	2.0×10^5
III	5.0×10^4
IV	1.0×10^5
V	1.4×10^5

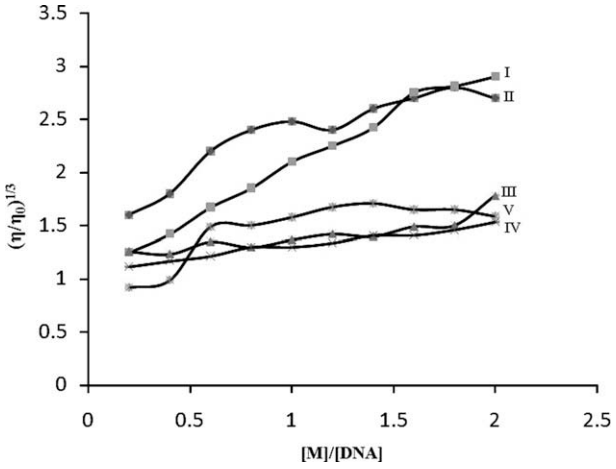


Figure 3. The effect of the complexes on the viscosity of pBR322 DNA.

against tested organisms suggest further investigation on these compounds.[19]

To explore the interaction between complexes and DNA, we carried out absorption titration measurements. With increasing concentrations of DNA, for complexes I–V, the observed data were then utilized to obtain the intrinsic binding constant, K_b using the following equation:

$$\frac{[DNA]}{(\epsilon_a - \epsilon_f)} = \frac{[DNA]}{(\epsilon_b - \epsilon_f)} + \frac{1}{k_b(\epsilon_b - \epsilon_f)} \quad (1)$$

where [DNA] is the concentration of DNA in terms of nucleotide phosphate, [NP] the apparent absorption coefficient ε_f, ε_a, and ε_b correspond to the extinction coefficient of the free complex, the

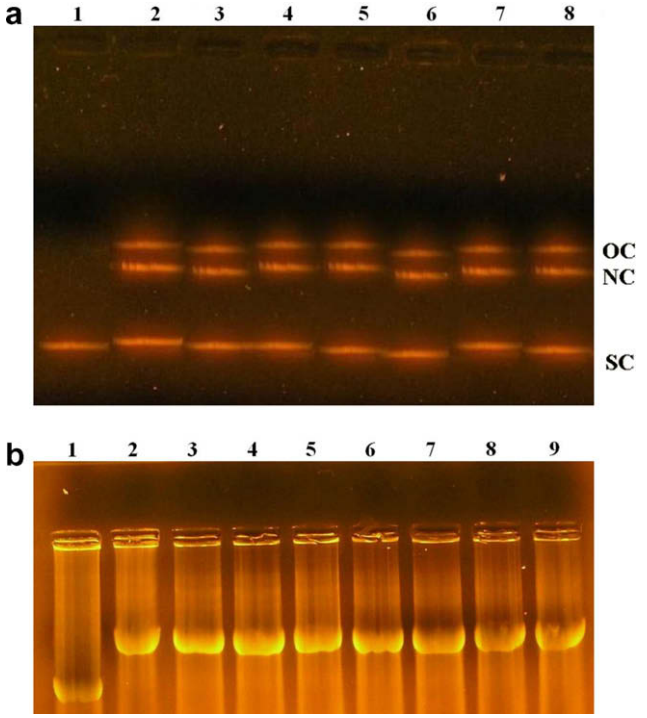


Figure 4. (a) Gel electrophoresis data with pBR322 DNA. Lane 1: pBR322 (Control), Lane 2: pBR322 + $VOSO_4$, Lane 3: pBR322 + Cip, Lane 4: pBR322 + I, Lane 5: pBR322 + II, Lane 6: pBR322 + III, Lane 7: pBR322 + IV, Lane 8: pBR322 + V. (b) Gel electrophoresis data with pBR322 DNA. Lane 1: pBR322 (Control), Lane 2: pBR322 + H_2O_2, Lane 3: pBR322 + H_2O_2 + $VOSO_4$, Lane 4: pBR322 + H_2O_2 + Cip, Lane 5: pBR322 + H_2O_2 + I, Lane 6: pBR322 + H_2O_2 + II, Lane 7: pBR322 + H_2O_2 + III, Lane 8: pBR322 + H_2O_2 + IV, Lane 9: pBR322 + H_2O_2 + V.

Table 6
Gel electrophoresis data of the complexes

Compounds	% (SC)	% (NC)	% (OC)
DNA Control	100	—	—
DNA + $VOSO_4$	52	23	25
DNA + Cip	56	21	23
DNA + I	38	32	30
DNA + II	34	36	30
DNA + III	30	36	34
DNA + IV	34	31	35
DNA + V	32	34	34

Table 7
Suppression ratio data of the complexes

Complexes	Suppression ratio
I	−9.8
II	−8
III	−5.6
IV	−9.6
V	−7.4

extinction coefficient for each addition of DNA to the complex and the extinction coefficient for the complex in the fully bound form, respectively, and K_b is the ratio of the slope to the y intercept. The application of electronic absorption spectroscopy in DNA-binding studies is one of the most useful techniques.[20] Complex binding with DNA through intercalation usually results in hypsochromism and bathochromism, because intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The absorption spectra of the complex in the absence and presence of DNA are illustrated in Figure 2. Change in absorbance at the peak maximum shows moderate hypsochromism shift (~2 nm). For each complex, increasing concentration of DNA has been monitored for an evaluation of the intrinsic binding constant, which was in the range of 5.0×10^4–2.0×10^5 M^{-1} (Fig. 2, inset, for the plot for the calculation of intrinsic binding constant). The binding constants are given in Table 5. These spectral characteristics are consistent with a mode of interaction that involves a stacking interaction between the complex and the base pairs of DNA, which means that the titled complexes can intercalate into the double helix structure of DNA.

Viscosity data are presented as $(\eta/\eta_0)^{1/3}$ versus binding ratio,[21] where η_0 and η are the viscosity of DNA in the absence and presence of the complex, respectively.

Optical photophysical probes are necessary, but not sufficient clues to support a binding mode of small molecules to DNA. Further clarification of the interaction mode between the complexes and DNA is carried out by viscosity measurements. Hydrodynamic measurements that are sensitive to length change (i.e., viscosity and sedimentation) of DNA are regarded as the least ambiguous and the most critical tests of binding mode in solution in absence of crystallographic structural data.[22] A classical intercalation mode results in lengthening of the DNA helix, as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. The effect of the complexes on the viscosity of DNA is shown in Figure 3. It is found that the viscosity of DNA increases steadily with the increase of the concentration of the complex, which is similar to that of a classical intercalator EB.[23] This result demonstrates that the complexes and EB bind to DNA through the same way, i.e., the classical intercalation mode. The significant increase in viscosity of the complexes is obviously due to the partial insertion of the ligand in between the DNA base pairs leading to increase in separation of base pairs at intercalation sites and hence an increase in overall DNA contour length.[24]

The characterization of DNA recognition by transition metal complexes has been aided by the DNA cleavage chemistry that is associated with redox-active or photoactivated metal complexes. DNA cleavage is controlled by relaxation of supercoiled circular form (I) of pBR322 DNA into nicked circular form (II) and linear form (III). Fig. 4a and b illustrates the gel electrophoretic separations showing the cleavage of plasmid pBR322 DNA induced by the complexes under aerobic conditions and in the presence of H_2O_2, respectively.[25] When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (SC). If one strand is cleaved, the supercoil will relax to produce a slower-moving open-circular form (OC). If both strands are cleaved, a nicked form (NC) will be generated that migrates in between. This clearly shows that the relative binding efficacy of the complexes to DNA is much higher than the binding efficacy of metal salt itself or ciprofloxacin (Table 6). The different DNA cleavage efficiency of the complexes was due to the different binding affinity of the complexes to DNA, which has been observed in other cases. One of the most interesting electrophoretic results of the complexes takes place when experiment is done in presence of H_2O_2 in TAE buffer. The DNA + complex + H_2O_2 systems (Fig. 4a) cleave the supercoiled DNA form (I) and convert it into 100% linear form (III). Therefore, we concluded that DNA scission is observed due to the formation of hydroxyl radical as shown in following scheme:

$$2V^{4+} + O_2 + 2H^+ \rightarrow 2V^{5+} + H_2O_2$$
$$V^{4+} + H_2O_2 + H^+ \rightarrow V^{5+} + {}^{\cdot}OH + H_2O$$

The superoxide dismutase-like activity[26] of the complexes was determined (Table 7). The negative value of suppression ratio indicates the absence of oxygen radicals and formation of hydroxyl radicals. Which support the cleavage of supercoiled DNA in the presence of H_2O_2 is due to the formation of hydroxyl radical. The suppression ratio of the complexes is calculated using the following equation:

$$\text{Suppression ratio } (O_2^-) = 100[(A_0 - A_i)/A_0] \quad (2)$$

where, A_0 = absorbance in the absence of complex and A_i = absorbance in the presence of complex.

Acknowledgments

We express our gratitude to Prof. J. S. Parmar, Head, Department of Chemistry and Head, BRD School of Biosciences, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India, for providing the necessary laboratory facilities. The authors are highly thankful to UGC for financial assistance of UGC Grant 32-226/2006 (S.R.).

Table 8
Experimental and physical parameters of the complexes

Complexes empirical formula	% Found (required)				μ_{eff} (BM)	Mp (°C)	% Yield	Formula weight (g/mol)
	C	H	N	M				
$C_{20}H_{27}FN_4O_8V$ (I)	46.10 (46.07)	5.20 (5.22)	10.71 (10.75)	9.75 (9.77)	1.73	>360	62.5	521.39
$C_{26}H_{31}FN_4O_9V$ (II)	50.89 (50.90)	5.06 (5.09)	9.11 (9.13)	8.33 (8.30)	1.68	>360	65.2	613.49
$C_{28}H_{32}FN_5O_8V$ (III)	52.81 (52.83)	5.10 (5.07)	10.96 (11.00)	8.03 (8.00)	1.71	>360	64.5	636.52
$C_{22}H_{29}FN_4O_{10}V$ (IV)	45.58 (45.60)	5.01 (5.04)	9.66 (9.67)	8.81 (8.79)	1.71	>360	61.8	579.43
$C_{23}H_{33}FN_4O_8V$ (V)	49.04 (49.03)	5.93 (5.90)	9.89 (9.94)	9.06 (9.04)	1.70	>360	58.9	563.47

References and notes

1. Guo, Z.; Sadler, P. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1512.
2. Lippert, B. *Coord. Chem. Rev.* **2000**, *487*, 200.
3. Lippard, S. *J. Acc. Chem. Res.* **1978**, *11*, 211.
4. Barton, J. K. *Science* **1986**, *223*, 727.
5. Dervan, P. B. *Science* **1986**, *223*, 464.
6. Tonde, S. S.; Kumbhar, A. S.; Padhye, S. B.; Butcher, R. J. *J. Inorg. Biochem.* **2006**, *100*, 51.
7. Shen, L. L.; Pernet, A. G. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 307.
8. Permana, P. A.; Snapka, R. M.; Shen, L. L.; Chu, D. T. W.; Clement, J. J.; Platter, J. *J. Biochem.* **1994**, *33*, 11333.
9. Turel, I.; Leban, I.; Klintsebar, G.; Bukovec, N.; Zalar, S. *J. Inorg. Biochem.* **1997**, *63*, 76.
10. Patel, M. N.; Pansuriya, P. B. *Appl. Organometal. Chem.* **2007**, *21*, 739.
11. Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; ELBS and Longman: London, 2004.
12. Weiss, H. *Witte Magnetochemie*; Verlag Chemie: Weinheim, 1973.
13. Leban, I.; Turel, I.; Bukovec, N. *J. Inorg. Biochem.* **1997**, *66*, 241.
14. Dobrzyńska, D.; Jerzykiewicz, L. B.; Duczmal, M. *Polyhedron* **2005**, *24*, 407.
15. Silverstein, R. M.; Webster, F. X. *Spectrometric Identification of Organic Compounds*, 6th ed.; John Wiley and Sons, Inc.: New York, 2004.
16. Bhattacharya, S.; Mukhopadhyay, S.; Samanta, S.; Weakley, T. J. R.; Chaudhury, M. *Inorg. Chem.* **2002**, *41*, 2433.
17. Caravan, P.; Gelmini, L.; Glover, N.; Herring, F. G.; Li, H.; McNeill, J. H.; Rettig, S. J.; Setyawati, I. A.; Shuter, E.; Sun, Y.; Tracey, A. S.; Yuen, V. G.; Orvig, C. *J. Am. Chem. Soc.* **1995**, *117*, 12759.
18. Patel, S. H.; Pansuriya, P. B.; Chhasatia, M. R.; Parekh, H. M.; Patel, M. N. *J. Therm. Anal. Calorim.* **2008**, *91*, 413.
19. *Dilution method*: All the bacterial species were incubated and activated at 37 °C for 24 h by inoculating them to Luria broth. The compounds were dissolved in DMSO and then diluted using cautiously adjusted Luria broth. Twofold serial concentrations of the compounds were employed to determine the (MIC) ranging from 0.1 to 3000 μM. Test cultures were incubated at 37 °C (24 h). The lowest concentrations of antimicrobial agents that result in complete inhibition of microorganisms were represented as (MIC) micromolar. In each case triplicate tests were performed and the average was taken as the final reading. Jones, R. N.; Barry, A. L.; Gaven. T. L.; Washington, J. A.; Lennette, E. H.; Balows A.; Shadomy, W. J. *Manual of Clinical Microbiology*, 4th ed.; Am. Soc. Microb. Washington, DC, 1984, 972 pp.
20. *UV–vis spectroscopy:* The concentration of DNA was measured by using its standard extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}). The absorbance at 260 nm (A_{260}) and at 280 nm (A_{280}) was measured to check purity of DNA. The ratio of A_{260} to A_{280} was found to be 1.68, indicates that DNA was satisfactorily free from protein. Phosphate buffer (1 mM, pH 7.2) was used for the absorption titration experiment. Absorption titration was carried out by varying the DNA concentration (0–100 μM) and maintaining constant concentration of the complex (4 μM). Absorption spectra were recorded after each successive addition of DNA followed by allowing it to attain equilibrium (approximately 10 min). (a) Reichmann, M. E.; Rice, S. A.; Thomas, C. A.; Doty, P. *J. Am. Chem. Soc.* **1954**, *76*, 3047; (b) Wolfe, A.; Shimer, G. H.; Meehan, T. *Biochemistry* **1987**, *26*, 6392; (c) Barton, J. K.; Raphael, A. L. *J. Am. Chem. Soc.* **1984**, *106*, 2172; (d) Kelly, T. M.; Tossi, A. B.; McConnell, D. J.; Strekas, T. C. *Nucleic Acids Res.* **1985**, *13*, 6017; (e) Tysoe, S. A.; Morgan, R. J.; Baker, A. D.; Strekas, T. C. *J. Phys. Chem.* **1993**, *97*, 1707.
21. *Viscosity measurements:* Viscosity measurements were carried out using an Ubbelodhe viscometer maintained at a constant temperature 27.0 ± 0.1 °C in a thermostatic bath. Flow time was measured with a digital stopwatch in triplicate, and an average flow time was considered. Cohen, G.; Eisenberg, H. *Biopolymers* **1969**, *8*, 45.
22. (a) Satyanaryana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1993**, *32*, 2573; (b) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1992**, *31*, 9319.
23. Wang, X. L.; Chao, H.; Li, H.; Hong, X. L.; Liu, Y. J.; Tan, L. F.; Ji, L. N. *J. Inorg. Biochem.* **2004**, *98*, 1143.
24. (a) Baguley, B. C.; LeBret, M. *Biochemistry* **1984**, *23*, 937; (b) Lerman, L. *J. Mol. Biol.* **1961**, *3*, 18.
25. *Gel analysis and quantification:* The plasmid DNA pBR322 (4363 base pairs in length, density of supercoiling, $r = -0.065$), was prepared by transformation of pBR322 into safe competent cells (*E. coli* strain), amplification of a clone as outlined by Sambrook et al. After concentration by ethanol precipitation, DNA was stored in TE buffer (pH 8.0) at −20 °C. The relative amount of the supercoiled (SC) form was checked by gel electrophoresis on agarose. The preparations contained about 100% of the SC form and 0% of the open-circular (OC) form. Cleavage of pBR322 by VO(IV) complexes were accomplished by mixing (in order) TE buffer (pH 8.0), complexes (50 μM) and pBR322 (2.5 μg ml^{-1}). After mixing, the samples were incubated at room temperature for 30 min and loaded with 0.5× loading buffer containing 40% sucrose and 0.02% bromophenol blue on 1.5% agarose gel. DNA bands were then viewed with a transilluminator and the images captured with an attached camera and the extent of cleavage was determined via densitometry analysis of EtBr-stained agarose gel, by using the volume quantization AlphaDigiDoc™ RT. Version V.4.1.0 PC-Image software. The relative amounts of the different forms of DNA were determined by dividing the fluorescence intensity for a particular band by the sum of the fluorescence intensities for each band in that lane. Note that small differences in staining make exact quantitative gel-to-gel comparisons difficult. Densitometry is uncorrected for differential uptake of EtBr by SC and non-SC DNA. A previous study with pBR322 plasmid under similar conditions showed this factor was small. (a) Sambrook, J.; Fritsche, E. F.; Maniatis, T. Molecular Cloning: A Laboratory Manual, 2nd ed.; New York: Cold Spring Harbor, 1989; (b) Hertzberg, R. P.; Derwan, P. B. *Biochemistry* **1984**, *23*, 3934; (c) Santra, B. K.; Reddy, P. A. N.; Neelakanta, G.; Mahadevan, S.; Nethaji, M.; Chakravarty, A. R. J. Inorg. Biochemistry **2002**, *89*, 191; (d) Navarro, M.; Cisneros-Fajardo, E. J.; Sierralta, A.; Fernández- Mestre, M.; Silva, P.; Arrieche, D.; Marchá n, E. *J. Biol. Inorg. Chem*. **2003**, *8*, 401; (e) Hertzberg R. P.; Derwan, P. B. *Biochemistry* **1984**, *23*, 3934.
26. *Superoxide dismutase activity:* Measurement of SOD-mimic activity. The SOD-mimic activity of the VO(IV) complexes was evaluated according to Viswanathan et al., which was first proposed by McCord and Fridovich. This in vitro method is based on the competing reactions of the tested compounds and nitroblue tetrazolium (NBT), phenazine methosulphate with NADH solution in distilled water. The reaction product is a blue formazan compound. The decrease in concentration of NBT is proportional to the SOD-mimic activity of the tested compounds. The reaction solution consisted of a solution of tested compound in DMSO and NBT, phenazine methosulphate and NADH in distilled water. The formazan product was subsequently estimated at 560 nm using a Shimadzu UV–vis spectrophotometer. The actual concentration of formazan dye produced was measured. (a) Kakkar, P.; Das, B.; Viswanathan, P. N. *Indian J. Biochem. Biophys*. **1984**, *21*, 130; (b) McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1969**, *244*, 6049.

Endnote:*Synthesis of the complexes*: An ethanolic solution (50 mL.) of $VOSO_4{\cdot}3H_2O$ (0.6327 g, 5 mM) was added to an ethanolic solution of (50 mL). DL-alanine (L1) (0.2227 g, 5 mM), followed by accumulation of formerly primed solution of (Cip·HCl) (0.917 g, 5 mM) in water and pH of the reaction mixture was adjusted to 6.0–7.0 pH with dilute NaOH solution. The resulting green solution was refluxed with stirring for 7 h, and then heated on steam bath to evaporate up to half volume. The reaction mixture was kept for overnight at room temperature. A fine green color product was obtained. The obtained product was washed with ether and dried over vacuum desiccators. The compounds II–V were prepared according to the same method and their physicochemical parameters are summarized in Table 8.