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DNA interaction, cytotoxicity, antibacterial and antituberculosis activity of an oxovanadium(IV) complexes derived from fluoroquinolones and 4-hydroxy- 5-((4-hydroxyphenyl)diazenyl)thiazole-2(3H)-thione

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Abstract

Oxovanadium complexes (5a-5g) with fluoroquinolone ligands and azodye rhodanine (4-hydroxy-5-((4-hydroxyphenyl)diazenyl)thiazole-2(3H)-thione) were synthesized and the effect of different groups has been studied with various biological and medicinal activities. The characterization of the newly formed compounds was done by ESI-MS, UV–Vis, IR, ESR spectroscopy, elemental analysis and molar electric conductibility. The *in vitro* antibacterial activity against three gram negative and two gram positive microorganisms was studied and compared to the activity of the free ligand and it shows considerable results. The minimum inhibitory concentration (MIC) of the compounds against *Mycobacterium tuberculosis* was determined; amongst all 5c and 5d show potency. The *in vitro* cytotoxicity of the complexes was determined with brine shrimp bioassay and LD_{50} is in the range of 4-12µg/mL. The DNA binding activity was studied by UV-Vis titration and viscosity measurements, 5c has highest binding constant value ($6.91\times10^5\ M^{-1}$). DNA nuclease activity was studied by agarose gel electrophoresis method and compounds show 70-80 % of cleavage.

Introduction

Quinolones, a term commonly used for the quinolone carboxylic acids or 4-quinolones, are a group of synthetic antibacterial agents containing a 4-oxo-1,4-dihydroquinoline skeleton [1]. Diverse modifications of the skeleton based on structure–activity relationships (SARs) were made in order to isolate structurally related highly potent broad-spectrum antibacterial agents [2]. In this context, the introduction of a fluorine atom at position 6 and a piperazine ring at position 7 has led to a great enhancement of the activity spectrum. Fluoroquinolones are extremely useful for the treatment of urinary tract infections, soft tissue infections, respiratory infections, typhoid fever, sexually transmitted diseases, bone-joint infections, prostatitis, community-acquired pneumonia, acute bronchitis, and sinusitis [2]. The activity of quinolones as antibacterial drugs is mainly due to the effective inhibition of DNA replication [1].

Vanadium is a trace bioelement with interesting biological properties [3]. Vanadium is essential in chlorophyll synthesis and it is present at the active site of several enzymes [4]. Several functions have been shown to be mimicked by simple vanadium salts as well as by oxovanadium(IV) species coordinated to organic ligands [5, 6]. The coordination chemistry of square pyramidal metal complexes involving nitrogen donor ligands has excited great interest among chemists in recent years due to applications in catalysis and their relevance to bioinorganic system [7]. The study of the interaction of the fluoroquinolones with diverse metal ions has been initiated [8] in an attempt to examine the mode of binding and possible synergetic effects. Keeping in view the significant bioactive nature of fluoroquinolones and nitrogen donor ligands as well as biological functioning of vanadium metal, it was thought valuable to merge the chemistry of organic ligands with the vanadium metal to form a novel class of vanadium metal based mixed ligand complexes that could serve as potential antibacterial agent against resistant bacterial strains. In this paper, we report the synthesis and characterization of the mononuclear VO(IV) complex **(5a-5g)** with seven different fluoroquinolones and azodye rhodanine ligand. The interaction of the complex with calf-thymus (CT) DNA has been also investigated with UV-Vis spectroscopy and antimicrobial efficiency of the complex has been evaluated by determining the minimum inhibitory concentration (MIC) against five different microorganisms. The cytotoxicity of oxovanadium(IV) complexes evaluated with brine shrimp bioassay. All the complexes applied for *Mycobacterium tuberculosis* H37Rv strain to check antituberculosis activity.

Chemistry

Materials for synthesis

Commercially obtained chemicals were used as received. Fluoroquinolones and rhodanine were purchased from Aldrich. $VOSO_4$ is purchased from Himedia lab. Agar powder, nutrient broth, sea salt were obtained from Himedia.

2.2. Instrumentation

Elemental analyses (C, H and N) of the synthesized complexes were performed with a model 240 Perkin Elmer elemental analyzer, Massachusetts (USA). Infrared spectra were recorded

on a FT-IR ABB Bomen MB-3000 spectrophotometer (Canada) as KBr pellets in the range 4000–400 cm^{-1}. The reflectance spectra of the metal complexes were recorded on UV-160A UV–visible spectrophotometer, Shimadzu, Kyoto (Japan). Room temperature magnetic measurement for the complexes was made using Gouy magnetic balance. The Gouy tube was calibrated using mercury(II) tetrathiocyanatocobaltate(II) as the calibrant ($\chi g=16.44\times10^{-6}$ cgs units at 20 °C). A Gallenkamp melting point apparatus (temperature range, 0–350 °C) was used to determine the melting point. Thermal stability of the materials was studied using a Perkin-Elmer TGA thermo gravimetric analyzer. The heating rate was suitably maintained at 10 °C min^{-1} under a nitrogen atmosphere/static air from 50 °C temperature up to 900 °C. EPR spectra were recorded with a Bruker ESP 300E X-band spectrometer. The ESI-MS were recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer using DMSO as the solvent. Antibacterial activity was performed using incubator and laminar air flow cabinet (Toshiba, Delhi, India). Photo quantization of the DNA nuclease activity was done using AlphaDigiDocTM RT. Version V.4.0.0 PC– Image software, CA (USA).

2.3 Synthesis of 4-hydroxy-5-((4-hydroxyphenyl)diazenyl)thiazole-2(3H)-thione (3)

In a typical preparation, 25 mL of distilled water containing 0.01 M hydrochloric acid were added to 4-hydroxyaniline [9-13]. The resulting mixture was stirred and cooled to 0℃, a solution of 0.01mol sodium nitrite in 20 mL of water was added dropwise. The formed diazonium chloride was consecutively coupled with an alkaline solution of 0.01mol 2-thioxo- 4-thiazolidinone (rhodanine) in 10 mL of pyridine. The formed coloured precipitate was filtered through sintered glass crucible, washed several times with water. The crude product was purified by recrystallization from hot ethanol; it yields 70% of product then dried in vacuum. The spectrums are given in supplementary material 1. Yield: 70 %; mp: 210 °C; mol. wt. 253.30, ^{13}C NMR (100 MHz, DMSO-d_6) δ: 192.95 (C-6), 182.90 (C-7), 154.36 (C-1), 73.29 (C-5) 135.87 (C-4), 116.74, 111.55 (4C, C-2, 2”, C-3, 3”); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 13.63 (s, 1H, NH), 10.85 (s, 1H, =C-OH), 9.27 (s, 1H, Ar-OH), 7.13 (dd, J=6.8 Hz, 2H, Ar–H), 6.76 (d, J=6.8 Hz, 2H, Ar–H). Anal. Calc. for: $C_9H_7N_3O_2S_2$, Calc. (Found) (%): C, 42.68 (42.60); H, 2.79 (2.45); N, 16.59 (16.35).

2.4. Synthesis of oxovanadium(IV) complexes (5a-5g)

2.4.1 [VO(GFL)(L)] [5a]

To a hot solution of $VOSO_4$ (5 mmol) in MeOH (25.0 mL), previously prepared methanolic solution of 4-hydroxy-5-((4-hydroxyphenyl)diazenyl)thiazole-2(3H)-thione (**3**) (5 mmol) was added, with constant stirring. Then alkaline methanolic solution of ciprofloxacin (5 mmol) was added. The pH of the reaction mixture was adjusted to ~6.8. The resulting brownish solution was refluxed for ten hours and concentrated under vacuum. Upon addition of Et_2O, a green solid precipitate was obtained, which was collected by filtration, washed with Et_2O and dried in vacuo[14]. Synthesis of complex is shown in Scheme 1. Yield: 62.61%. M.P: >300. Anal. Calcd (%). for $C_{26}H_{23}FN_6O_6S_2V$: C, 48.07; H, 3.57; N, 12.94; V, 7.84. Found (%): C, 47.70; H, 3.21; N, 12.52; V, 7.56. UV–Vis in DMSO [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 498 (20,080), 700 (14,285). FT-IR: υ_{max} (cm^{-1}) ν(C═O)pyridone 1631(vs), ν(CO_2)asym 1576(vs), ν(CO_2)sym 1373(vs), ν = ν(CO_2)asym-ν(CO_2)sym = 203, ν(V=O) 951(vs), ν(M-O) 510 cm^{-1}. ESI-MS (*m/z*):649.57 [M^+].

2.4.2 [VO(GFL)(L)] [5b]

The complex was prepared using a procedure similar to that for 2.2.1 using gatifloxacin (GFLH) instead of ciprofloxacin. Yield: 62.00%. M.P: >300. Anal. Calcd (%). for $C_{28}H_{27}FN_6O_7S_2V$: C, 48.48; H, 3.92; N, 12.12; V, 7.34. Found (%): C, 48.02; H, 3.67; N, 12.47; V, 7.69. UV–Vis in DMSO [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 502 (19,920), 701 (14,265). FT-IR: υ_{max} (cm^{-1}) ν(C═O)pyridone 1628(vs), ν(CO_2)asym 1589(vs), ν(CO_2)sym 1382(vs), ν = ν(CO_2)asym-ν(CO_2)sym = 207, ν(V=O) 950(vs), ν(M-O) 511 cm^{-1}. ESI-MS *m/z* (%): 693.62 [M^+].

2.4.3 [VO(LFL)(L)] [5c]

The complex was prepared using a procedure similar to that for 2.2.1 using levofloxacin (LFLH) instead of ciprofloxacin. Yield: 64.26%. M.P: >300. Anal. Calcd (%). for $C_{27}H_{25}FN_6O_7S_2V$: C, 47.72; H, 3.71; N, 12.37; V, 7.50. Found (%): C, 47.26; H, 3.60; N, 12.33; V, 7.80. UV–Vis in DMSO [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 506 (19,762), 712 (14,044). FT-IR: ν_{max} (cm^{-1}) ν(C═O)pyridone 1622(vs), ν(CO_2)asym 1581(vs), ν(CO_2)sym 1378(vs), ν = ν(CO_2)asym-ν(CO_2)sym = 203, ν(V=O) 953(vs), ν(M-O) 523 cm^{-1}. ESI-MS *m/z* (%): 679.59 [M^+].

2.4.4 [VO(OFL)(L)] [5d]

The complex was prepared using a procedure similar to that for 2.2.1 using ofloxacin (OFLH) instead of ciprofloxacin. Yield: 64.18%. M.P: >300. Anal. Calcd (%). for $C_{27}H_{25}FN_6O_7S_2V$: C, 47.72; H, 3.71; N, 12.37; V, 7.50. Found (%): C, 47.49; H, 3.98; N, 12.46; V, 7.48. UV–Vis in DMSO [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 510 (19,607), 710 (14,084). FT-IR: ν_{max} (cm^{-1}) ν(C═O)pyridone 1631(vs), ν(CO_2)asym 1591(vs), ν(CO_2)sym 1386(vs), ν = ν(CO_2)asym-ν(CO_2)sym = 205, ν(V=O) 952(vs), ν(M-O) 520 cm^{-1}. ESI-MS *m/z* (%): 679.59 [M^+].

2.4.5 [VO(SFL)(L)] [5e]

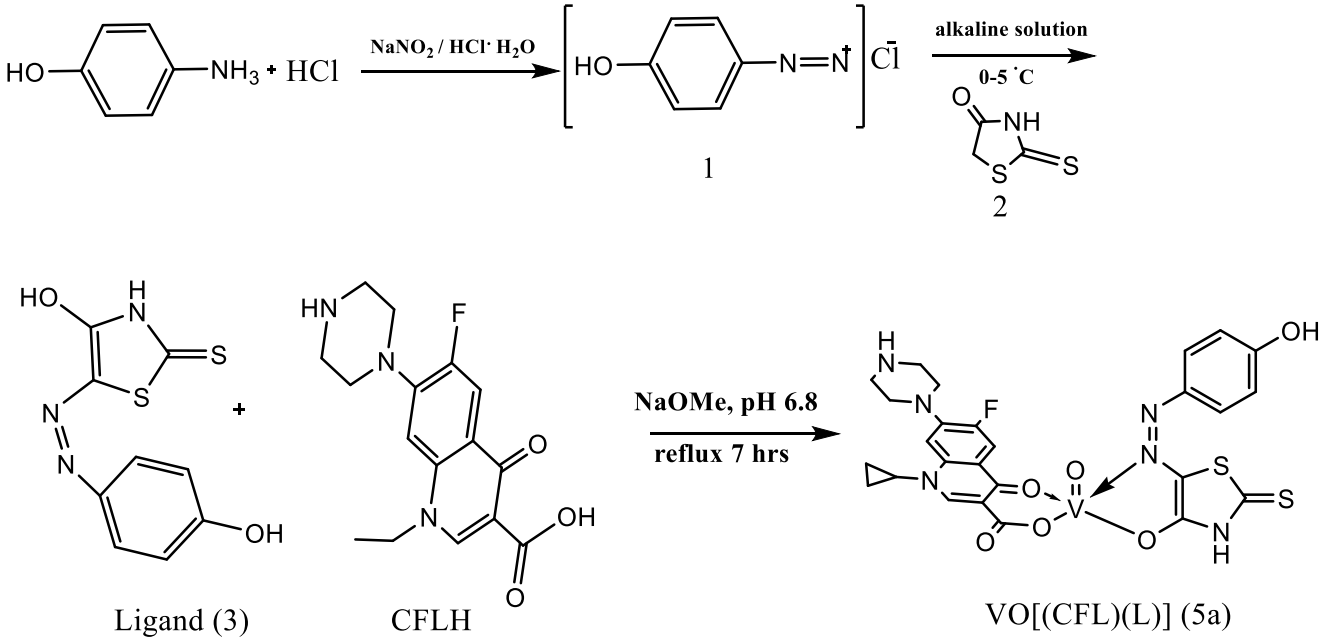
The complex was prepared using a procedure similar to that for 2.2.1 using sparfloxacin (SFLH) instead of ciprofloxacin. Yield: 61.48%. M.P: >300. Anal. Calcd (%). for $C_{28}H_{27}F_2N_7O_6S_2V$: C, 47.32; H, 3.83; N, 13.80; V, 7.17. Found (%): C, 47.56; H, 3.51; N, 13.46; V, 7.33. UV–Vis in DMSO [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 518 (19,305), 723 (13,831). FT-IR: ν_{max} (cm^{-1}) ν(C═O)pyridone 1639(vs), ν(CO_2)asym 1587(vs), ν(CO_2)sym 1381(vs), ν = ν(CO_2)asym-ν(CO_2)sym = 206, ν(V=O) 947(vs), ν(M-O) 521 cm^{-1}. ESI-MS *m/z* (%): 710.63 [M^+].

2.4.6 [VO(NFL)(L)] [5f]

The complex was prepared using a procedure similar to that for 2.2.1 using norfloxacin (NFLH) instead of ciprofloxacin. Yield: 59.77%. M.P: >300. Anal. Calcd (%). for $C_{25}H_{23}FN_6O_6S_2V$: C, 47.10; H, 3.64; N, 13.18; V, 7.99. Found (%): C, 47.12; H, 3.90; N, 13.56; V, 8.02. UV–Vis in DMSO [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 519 (19,267), 721 (13,869). FT-IR: ν_{max} (cm^{-1}) ν(C═O)pyridone 1624(vs), ν(CO_2)asym 1585(vs), ν(CO_2)sym 1378(vs), ν = ν(CO_2)asym-ν(CO_2)sym = 207, ν(V=O) 945(vs), ν(M-O) 516 cm^{-1}. ESI-MS *m/z* (%): 637.56 [M^+].

2.4.7 [VO(PFL)(L)] [5g]

The complex was prepared using a procedure similar to that for 2.2.1 using pefloxacin (PFLH) instead of ciprofloxacin Yield:



Scheme 1: Synthesis of 4-hydroxy-5-((4-hydroxyphenyl)diazenyl)thiazole-2(3H)-thione and complex 5a

57.32%. M.P: >300. Anal. Calcd (%). for $C_{24}H_{21}FN_6O_6S_2V$: C, 46.23; H, 3.39; N, 13.48; V, 8.17. Found (%): C, 46.28; H, 3.69; N, 13.67; V, 8.15. UV–Vis in DMSO [λ_{max}/nm (ε/M^{-1} cm^{-1})]: 513 (19,493), 718 (13,927). FT-IR: ν_{max} (cm^{-1}) ν(C═O)pyridone 1626(vs), ν(CO_2)asym 1589(vs), ν(CO_2)sym 1378(vs), ν = ν(CO_2)asym-ν(CO_2)sym = 205, ν(V=O) 948(vs), ν(M-O) 519 cm^{-1}. ESI-MS *m*/*z* (%): 623.53 [M^+].

2.5 Biological studies

2.5.1. *In vitro* cytotoxicity bioassay

The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method. Brine shrimp (Artemia cysts) eggs were hatched in a shallow rectangular plastic dish (22 - 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the matter compartment was opened to ordinary light. After two days nauplii was collected from the lighted side. The test samples were prepared by dissolving in DMSO (not more than 50 µL in 2.5 mL solution) and sea water. A vial containing 50 µL DMSO diluted to 2.5 mL was used as a control. 1 mL of seawater and 10 shrimps were added to each of 18 vials (3 set for each of 2, 4, 6, 8, 12, 16 and 20µg/mL) and the volume was adjusted with artificial seawater to 2.5 mL per vial. After 24 h the numbers of survivors were counted. The lethal concentrations of compounds resulting in 50% mortality of the brine shrimp (LD_{50}) from the 24 h and the dose–response data were transformed into a straight line by means of a trend line fit linear regression analysis (MS Excel version 10); the LD_{50} was determine from the best-fit line obtained [15, 16].

2.5.2 *In vitro* antibacterial activity

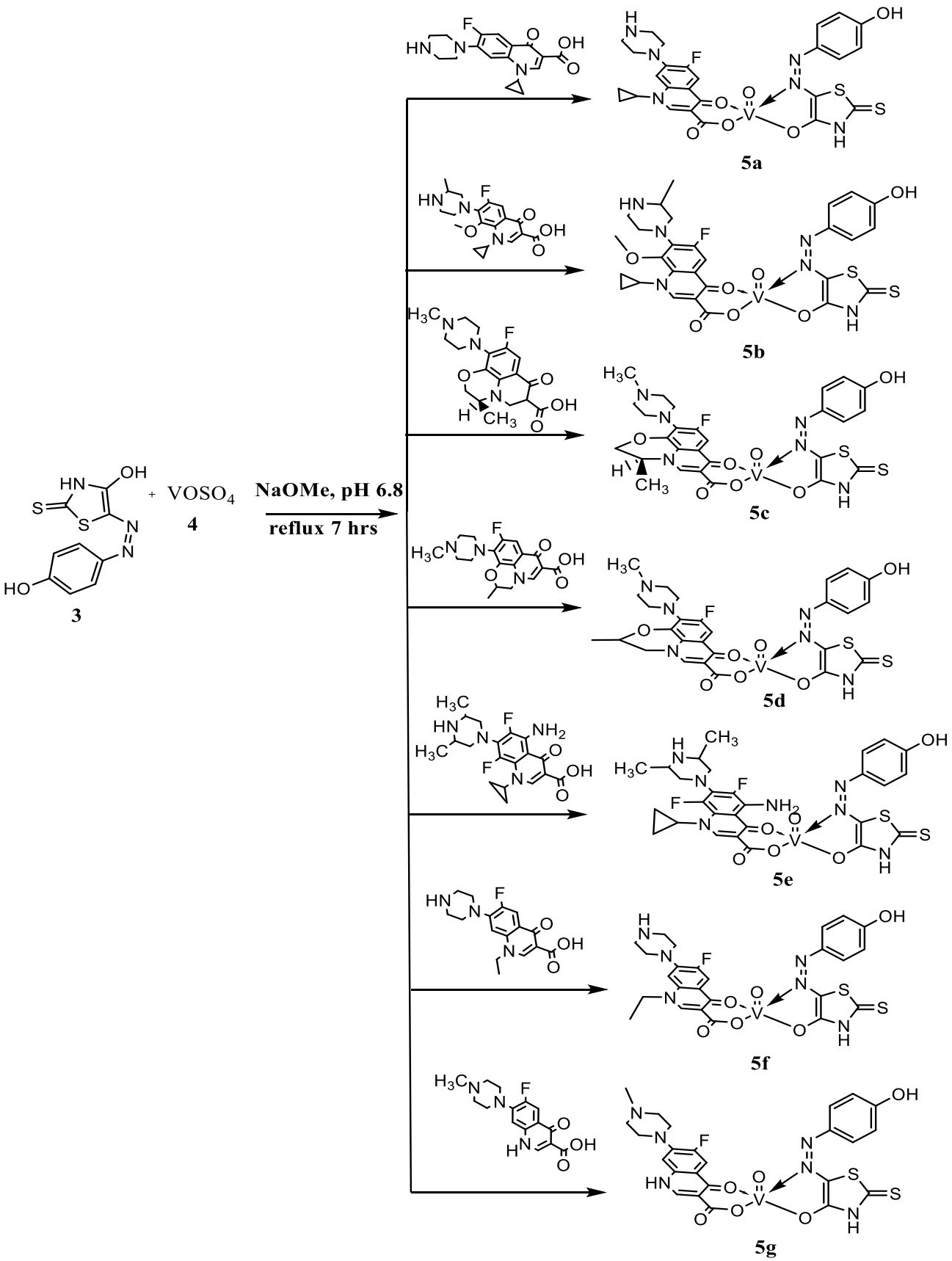
All newly synthesized oxovanadium (IV) complexes (5a-5g) were screened *in vitro* for their antibacterial activity against three Gram-negative microorganisms namely *Escherichia coli* (MTCC 433), *Pseudomonas aeruginosa* (MTCC P-09), *Serratia marcescens* (MTCC 7103) and two Gram-positive bacteria namely *Bacillus subtilis* (MTCC 7193) and *Staphylococcus aureus* (MTCC 3160). The antibacterial activity for the test compounds was performed to determine the bacteriostatic concentration, i.e. minimum inhibitory concentration (MIC). The MIC value was determined by two fold serial dilution technique in triplicate [17].

2.5.3 DNA binding experiments

CT-DNA was used for binding studies with the various oxovanadium mixed-ligand complexes investigated during this work. The stock solution was prepared by dissolving CT-DNA in a Tris HCl buffer (pH 7.2) and kept overnight at 4 °C for complete dissolution. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [18].

2.5.4 Viscosity measurements

Viscosity experiments were carried out using Cannon-Ubbelohde viscometer maintained at 27 °C in a thermostatic water bath. The flow time of solutions in a Tris HCl buffer (pH 7.2) was recorded in triplicate for each sample with a digital stopwatch, and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the binding ratio, where η is the viscosity of DNA in the presence of a complex and η_0 is the viscosity of DNA alone.



Scheme 2: General synthesis of oxovanadium(IV) complexes (5a-5g).

Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for that of buffer alone (t_0), $\eta \alpha t - t_0$.

2.5.5 DNA cleavage studies using agarose gel electrophoresis

Electrophoresis through agarose is the standard method used to separate, identify, or purify DNA fragments. When an electric field is applied across the gel, DNA, which is negatively charged at neutral pH, migrates toward the anode. This technique has been employed to identify the products of DNA cleavage, which was carried out in this work. A 10 µL total sample volume in 0.5 mL transparent eppendorf microtubes contains pUC19 DNA and metal complex. For the gel electrophoresis experiments, supercoiled pUC19 DNA was treated with metal complex and mixture was incubated for 30 min at 37 °C. The samples were analysed by 1% agarose gel electrophoresis [Tris-acetate-ethylenediaminetetraacetic acid (EDTA) (TAE) buffer, pH 8.0] for 3 h at 100 mV. The gel was stained with 0.5 µg/mL ethidium bromide, visualized by UV light and photographed for analysis. The extent of cleavage of the SC DNA was determined by measuring the intensities of the bands using AlphaDigiDocTM RT.

2.5.6 *In vitro* antituberculosis activity

All the synthesized complexes (5a-5g) were evaluated for their in vitro antituberculosis activity against the *Mycobacterium tuberculosis* H37Rv strain. Minimum inhibitory concentration of all the newly synthesized compounds was conducted using Lowenstein–Jensen medium as described by Rattan [19].

3. Results and discussion

3.1. Spectroscopic characterization of complexes (5a-5g)

3.1.1 IR spectra

In the IR spectrum of fluoroquinolone, the band at 1733 cm^{-1} is attributed to the absorption of the $\nu(C=O)_{carb}$ has been disappeared in oxovanadium complexes. Instead, two very strong characteristic bands are present in the range of 1576-1591 cm^{-1} and 1373-1386 cm^{-1} that could be assigned as ν(O–C–O) asymmetric and symmetric stretching vibrations, respectively[20], whereas $\nu(C=O)_{pyridone}$ is slightly shifted from 1622 to 1631 cm^{-1} upon bonding [21]. The difference $\Delta = \nu_{asym}(CO_2) - \nu_{sym}(CO_2)$, a useful characteristic for determining the coordination mode of carboxylate ligands, is about 203-207 cm^{-1} indicating a monodentate coordination mode of the carboxylato group . These changes of the IR spectra suggest that the fluoroquinolone ligand is coordinated to the metal via the $O_{pyridone}$ and one $O_{carboxylate}$ oxygen atoms [8]. The existence of ν(V=O) absorptions is a useful diagnostic tool for the characterization of the complex. The appearance of the V=O stretching frequency of complexes in the range of 945- 953 cm^{-1}, implies that a monoanionic ligand lies in trans-position to the $O_{vanadyl}$ [22]. The vibration of ν(M-N) observed at 530 [23]; ν(M-O) observed at 420-430 cm^{-1} [24] and 1483 cm^{-1} attributed to (N-H) vibration [25] (Table 1).

3.1.2 Mass spectra

The electron spray ionisation mass spectrum (ESI-MS) of complex (5a) possessed molecular ion peak at m/z 649.57; which is equivalent to its molecular mass and the fragment in the mass spectrum lead to the species $[C_{17}H_{17}FN_3O_4V]^+$ and $[C_9H_6N_3O_3S_2V]^+$. Some other fragments are observed at m/z = 397.27, 319.23, 330.33 and 252.29 respectively. The proposed mass fragmentation pattern of complex has been shown in Fig 1.

3.1.3 Electronic spectra

The electronic absorption spectra of the complexes were recorded for freshly prepared solution in DMSO at room temperature. Electronic absorption spectra of oxovanadium complexes show two d–d bands. The first band at ≈ 14250 cm^{-1} can be attributed to a d-d transition ($d_{xy} \rightarrow d_{xz;\ yz}$). The second band at ≈ 20250 cm^{-1} can be assigned to a $d_{xy} \rightarrow d_{x^2-y^2}$ transition, which is in consistent with that of square pyramidal geometry [26]. The magnetic moment values (Table 1) are found in the range 1.70–1.73 slightly less than the spin only value [27, 28].

3.1.4 ESR spectra

The ESR spectrum of VO(IV) (5a) complex has been studied under room temperature using TCNE as a g marker. The spectrum is a typical eight-line pattern, which shows that a single vanadium is present in the molecule, i.e., it is a monomer. The $g_{\parallel}$ and $g_{\perp}$ values have been found to be 2.0152 and 2.0617, respectively. The g_{av} was calculated to be 2.0457. The parameter G is found to be 0.217. The observed order of the parameters ($g_{\perp} > g_{\parallel}$) indicates that the unpaired electron is present in $(d_{xy})^1$ orbital with a square-pyramidal geometry around the oxovanadium(IV) complexes [29, 30]. The EPR spectrum of compound 5a is given in supplementary material 4.

3.2 Thermal studies

Room temperature stability for the complex (5a) was revealed by its thermogravimetric analysis, carried out from ambient temperature to 700 °C in nitrogen atmosphere. Complex shows no weight loss firstly in the range 30–200 °C. The mass loss at this temperature range may be corresponds to elimination of lattice H_2O molecules which is not present in the complex. So, the complexes do not possess octahedral geometry. The first decomposition peak starts at 225 °C is corresponds to organic moiety i.e. CFL (calc. / found: 51.09/50.27%), which ends at 423 °C. Further, the second decomposition step occurred in the temperature range 575– 775 °C is corresponding to another organic moiety, L (calc. / found: 39.02/37.97%) and leaving metal oxide behind. These observations further support the composition of complex, which is in agreement with a result obtained by elemental analysis studies.

3.2 Conductance and magnetic moment measurements of complexes (5a-5g)

The molar conductance values of the complexes (5a-5g) were taken in DMSO (Table 1). All he compounds show their conductance in the range 8.7-20.3 $\Omega^{-1}cm^2mol^{-1}$ showing nonelectrolytic nature [31, 32]. The magnetic moment values (1.70-1.78 BM) found at room temperature (Table 1), were compatible to the reported values for the square-pyramidal geometry [32, 33].

3.3 *In vitro* cytotoxicity bioassay

All the synthesized oxovanadium(IV) complexes (5a-5g) were screened for their cytotoxicity (brine shrimp bioassay) using Mayer et al. protocol [34, 35]. It was clearly evident from the studied data that only the complexes possessed potent cytotoxicity rather than the parent fluoroquinolones. Cytotoxicity of fluoroquinolones come in the range of 170-250 µg/mL as discussed in our previous work[36], which is too much higher than synthesized complexes (4-12 µg/mL)[37] as shown in Fig 2.

Table-1: Conductivity, magnetic moments, electronic spectra and IR data of complexes.

Compound	Ω_M ($\Omega^{-1}cm^2mol^{-1}$)	B.M. (μ_{eff})	λ_{max} (cm^{-1})	IR (ν, cm^{-1})					
				$(C=O)_{pyridone}$	$(CO_2)_{asym}$	$(COO)_{sym}$	[a] Δ	(V=O)	(M-O)
5a	10.9	1.70	20,080 14,285	1631	1576	1373	203	951	510
5b	12.6	1.72	19,920 14,265	1628	1589	1382	207	950	511
5c	8.7	1.72	19,762 14,044	1622	1581	1378	203	953	523
5d	21.6	1.70	19,607 14,084	1631	1591	1386	205	952	520
5e	20.9	1.71	19,305 13,831	1639	1587	1381	206	947	521
5f	14.9	1.72	19,267 13,869	1624	1585	1378	207	945	516
5g	20.3	1.71	19,493 13,927	1626	1589	1384	205	948	519

[a] $\Delta = \nu(CO_2)_{asym} - \nu(CO_2)_{sym}$.

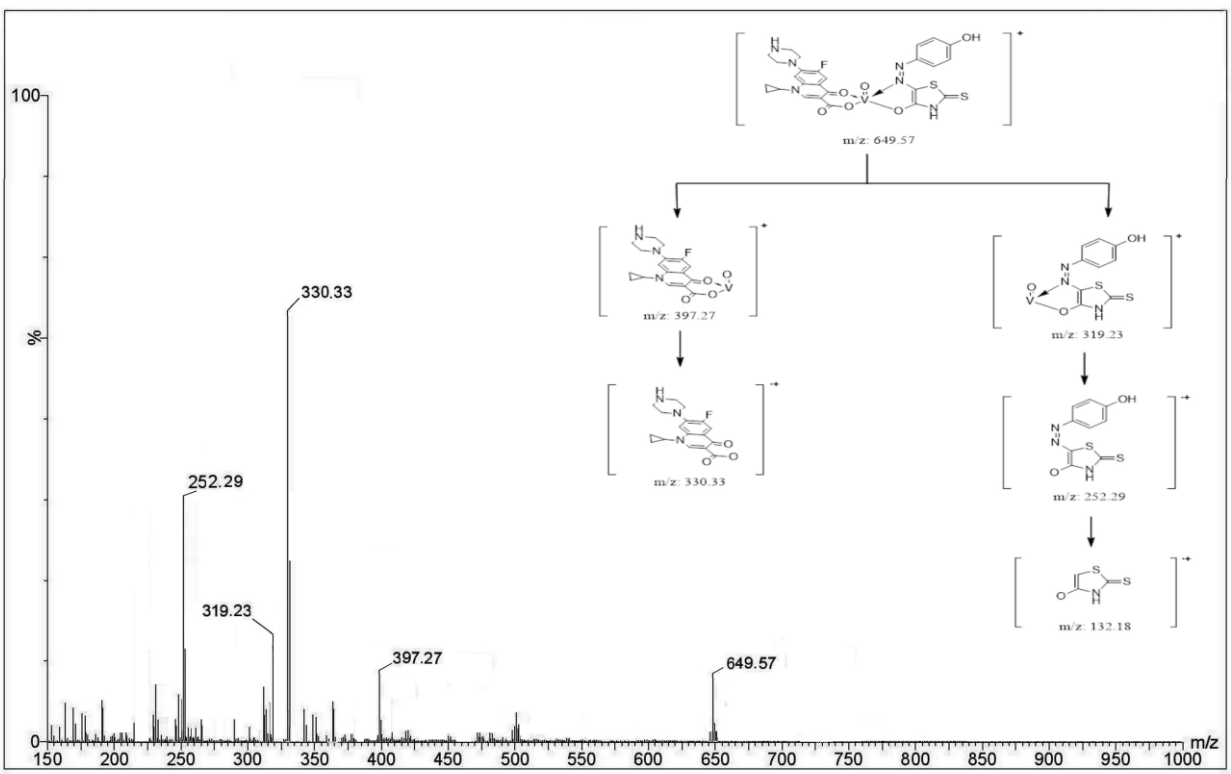


Fig. 1 LC-MS mass spectrum of complex 5a.

Clinically this bioassay relationship may help the researchers to serve as a basis for the future research work to design/develop the cytotoxic agents. The detailed results are shown in supplementary material 2.

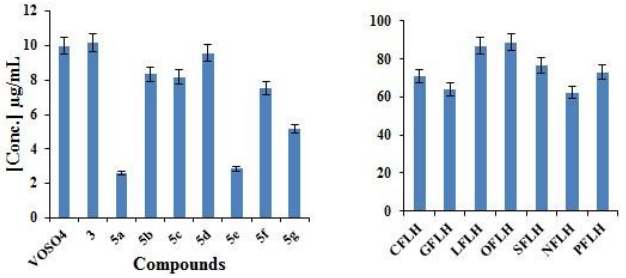


Fig. 2 Effect of compounds on brine shrimp lethality bioassay with error of ±5% for three replicates.

3.4 *In vitro* antibacterial activity

A comparative study of MIC values of the fluoroquinolone ligands and its complexes has been done. Present investigation of antimicrobial screening data (Table 2) reveal that all synthesized compounds exhibit good activities compared to free ligands, due to the greater lipophilic nature of the complexes. The increase in antibacterial activity of the metal chelates can be explained on the basis of chelating theory [38]. On chelating, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of the metal ion with donor groups. Further, it increases the delocalization of p-electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and blocks the metal binding sites on enzymes of micro-organisms[28].

Table 2: MIC studies of salt, fluoroquinolones and complexes with error of ±5% for three replicates.

Compound	Gram positive (in µM)		Gram negative (in µM)		
	S.A	***B.S***	***S.M***	***P.A***	***E.C***
$VOSO_4$	2568	2568	2568	1580	1185
CFLH	1.63	1.08	1.63	1.36	1.36
GFLH	5.06	3.99	2.93	1.01	2.93
LFLH	1.66	2.21	1.66	1.66	0.96
OFLH	1.93	1.38	1.66	2.21	1.38
SFLH	1.27	2.03	1.52	1.52	1.27
NFLH	2.50	2.50	4.07	3.75	2.81
PFLH	2.09	2.39	5.09	5.69	2.69
3	180	202	280	287	192
5a	0.7	0.9	1.6	1.3	1.3
5b	3.7	3.5	2.8	0.9	2.5
5c	0.3	1.8	1.4	1.4	0.8
5d	0.6	1.1	1.4	1.8	1.3
5e	0.3	1.3	1.2	1.3	1.0
5f	1.7	1.6	3.5	2.9	2.2
5g	1.1	1.4	3.3	3.7	2.3

S.A; *S. aureus,* ***B.S;*** *B. subtilis,* ***S.M;*** *S. marcescens,* ***P.A;*** *P. aeruginosa,* ***E.C;*** *E. coli*

3.5. DNA activity

3.5.1 Electronic absorption titration

DNA can provide three distinctive binding sites for quinolone–metal complexes; namely, groove binding, binding to phosphate group, and intercalation[39]. This behaviour is of great importance with regard to the relevant biological role of quinolone antibiotics in the body [40]. The UV–Vis titration of all complexes in absence and in presence of CT DNA has been studied in Tris buffer medium. The absorption spectra of 5c (at a constant concentration of complexes and wavelength range of 800–200 nm) are given in Fig. 3.

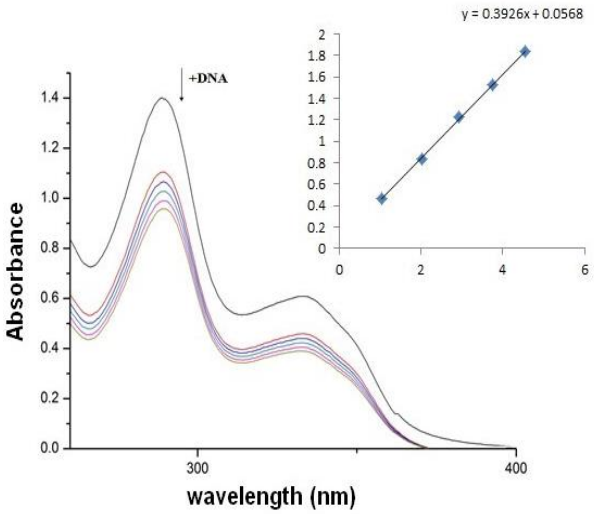


Fig.3 Electronic absorption spectra of complex 5c in Tris- HCL buffer (pH 7.2) in the absence and presence of increasing amount of CT-DNA. Inset: Plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *vs.* [DNA]. Arrow shows the absorbance change upon increasing DNA concentrations.

The absorption band of complex 5c at 288 nm exhibits hypochromism and bathochromism of about 3 nm. All other complexes also exhibit an evident hypochromism in the range of 270-290 nm and bathochromism of about 2-5 nm. These spectral characteristics suggest that all complexes interact with DNA most likely through a stacking mode of interaction between an aromatic chromophore and the base pairs of DNA. In order to further elucidate the binding strength of the complexes, the intrinsic binding constants K_b were calculated by monitoring the changes of absorbance in the ligand transfer bands with increasing amounts of CT DNA. The binding constant (k_b) values of complexes are calculated from equation

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}$$

and they are in the range of 0.22-6.91×10^5 M^{-1}. The K_b values of the complexes are slightly lower than those reported for the typical classical intercalators ethidium bromide (K_b, 1.4×10^6 M^{-1}) [41], and higher than those reported for vanadyl complexes [$C_{25}H_{17}N_3O_3V$ (4.2×10^4 M^{-1})] and [$(VO_2C_{14}H_{14}N_2O_2)_2SO_4$ (5.23 ×10^4 M^{-1})] [42, 43]. The detailed results are shown in supplementary material 2.

3.5.2 Viscosity measurements

Furthermore, the interactions between the complexes and DNA were investigated by viscosity measurements. In the absence of

X-ray structural data, viscosity measurement is regarded as the most effective means to study intercalative binding mode of DNA in solution [44]. A classical intercalative mode causes significant increase in viscosity of the DNA solution due to an increase in separation of base pairs at the intercalation sites and hence an increase in overall DNA length. In contrast, a partial, non-classical intercalation of ligand could bend (or kink) the DNA helix, reduce its effective length and, concomitantly, its viscosity [45].

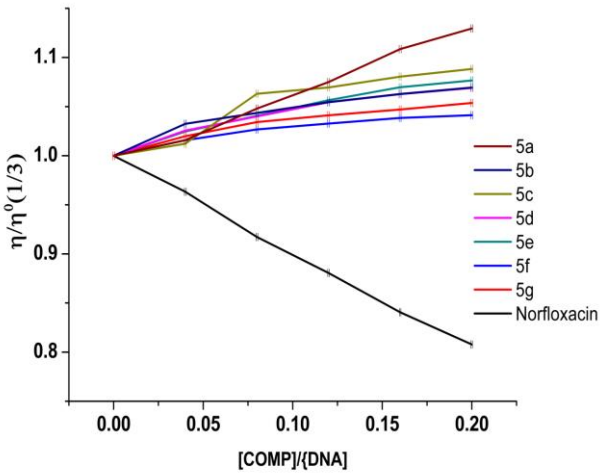


Fig. 4 Relative viscosity (±0.05) of CT- DNA under the influence of increasing amount of complexes at 27±0.1 °C in Tris- HCL buffer (pH 7.2).

As seen in Fig 5, the viscosity of CT DNA increases with increase in the ratio of complexes to CT DNA, also this result resemble the binding mode of ethidium bromide to CT DNA. The results were parallel to the above UV-spectroscopic data such as hypochromism and red-shift of complexes in the presence of DNA.

3.5.3 Electrophoretic analysis

There are a number of agents, which exert their effect by inhibiting enzymes that act upon DNA. These inhibitions result from binding of agents to the enzyme's site of interaction on the DNA and not direct enzyme inactivation. Transition metals have been seen to inhibit DNA repair enzymes. When pUC19 DNA is run on horizontal gel using electrophoresis, the fastest migration will be observed for the open circular form (Form I). If one strand cleaves a slower-moving linear form (Form II) will be observed. The gel electrophoretic separation of pUC19 DNA induced by metal complexes was conducted at 35℃ [46]. The difference was observed in the bands of complexes (Lane 3–9) compare to the control pUC19 DNA. Here, I, II and III indicates SC, NC and Linear form of DNA. It shows that the control DNA alone does not show any apparent cleavage whereas complexes shown. The results indicate the important role of metal in the isolated DNA cleavage reactions. As the compounds are observed to cleave the DNA, it can be conclude that the compound inhibits the growth of the pathogenic organism by cleaving the genome [47-49].The complexes show cleavage in the range of 75-85%. The detailed results are shown in supplementary material 3.

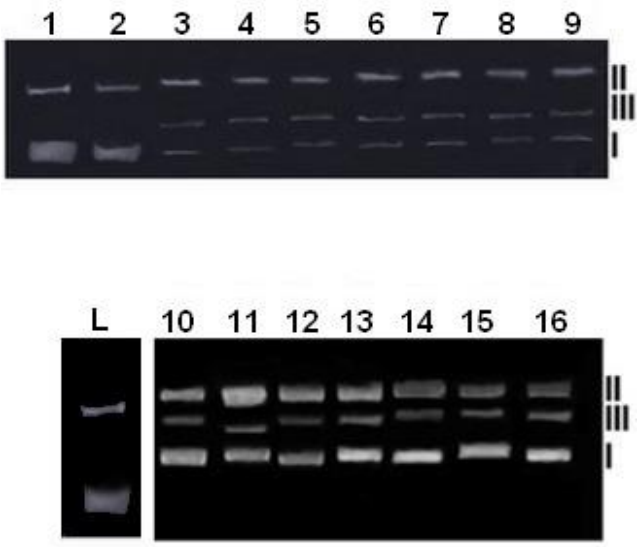


Fig. 5 Photogenic view of cleavage of pUC19 DNA (300 µg/mL) with series of oxovanadium(IV) complexes (200 µM) using 1% agarose gel containing 0.5 µg/mL ethidium bromide. All reactions were incubated in TE buffer (pH 8) in a final volume of 15 µL, for 24 h. at 37 °C. Form I, SC; Form II, NC; Form III, Linear and Lane 1, DNA control; Lane 2, $VOSO_4$; Lane 3, (5a); Lane 4, (5b); Lane 5, (5c); Lane 6, (5d); Lane 7, (5e); Lane 8, (5f); Lane 9, (5g); L, (compound 3); Lane 10 (CFLH); Lane 11, (GFLH); Lane 12, (LFLH); Lane 13, (OFLH); Lane14, (SFLH); Lane 15, (NFLH); Lane 16, (PFLH).

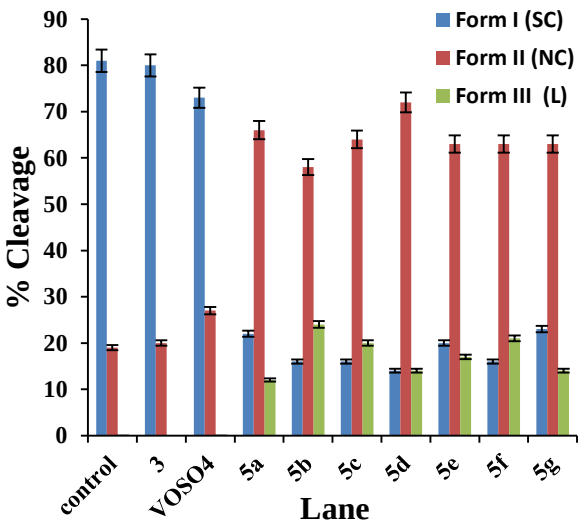


Fig. 6 Percentage cleavage (±3%) of SC, NC and Linear form of DNA.

3.6 *In vitro* antituberculosis activity

The cheering results from the antibacterial activity encouraged us to go for screening of all synthesized compounds for their *in vitro* antituberculosis activity. Antituberculosis screening of all complexes were conducted against *M. tuberculosis* H37Rv strain. Complexes **5c** and **5d** show better inhibition of *M. tuberculosis* growth than rest of all and as compared to fluoroquinolones. Isoniazid was used as the standard drugs. The results of antituberculosis screening data are shown in Table 3.

Table 3: Antituberculosis activity with error ±5% for three replicates.

Compound	MIC µg/mL
5a	250±12.5
5b	1000±50
5c	50±2.5
5d	25±1.25
5e	250±12.5
5f	500±25
5g	1000±50

Concluding remarks

The VO(IV) were coordinated to a deprotonated carboxylate oxygen and pyridone oxygen of fluoroquinolones to form a six-membered chelate ring with square pyramidal geometry. From the antibacterial activity data, it is observed that the complexes exhibit excellent activity against all five microorganisms compared with the free ligand, metal salt and the standard fluoroquinolone compounds. The increase in antibacterial activity of the complexes may be due to the metal chelation. The enhancement of the antibacterial activity is considered according to the kind of the metal ion that we used with the ligand. The effectiveness of the binding of complex is being confirmed by means of hypochromism in the electronic spectral study. Besides, the effectiveness of binding is also confirmed by the viscometric study. It shows that the complex interacts with DNA base pairs effectively, 5c gives highest binding constant compare to all other complexes due to planarity of levofloxacin The super-coil DNA is being cleave in the electrophoresis by the complex, which confirms that the complex having the ability to act as a potent DNA cleaving agent. The complex shows diverse cytotoxic and antituberculosis activities in comparison to the fluoroquinolones.

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Notes and references

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†Electronic Supplementary Information (ESI) available: [^{1}H NMR and ^{13}CMR spectra; DNA binding and cytotoxic data of fluoroquinolones and complexes 5a-5g; Gel electrophoresis studies of compounds.]

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