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DNA interaction, *in vitro* antimicrobial and SOD-like activity of copper(II) complexes with norfloxacin and terpyridines

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ABSTRACT

The novel octahedral copper(II) complexes with the second generation fluoroquinolone, norfloxacin and terpyridine derivatives were prepared and characterized. The antimicrobial efficiency of the complexes were tested on five different microorganisms and showed good biological activity. Viscosity measurement and absorption titration were employed to determine the mode of binding of complexes with DNA whereas the cleavage efficacy of the complexes towards pUC19 DNA was determined by electrophoresis in presence of ethidium bromide. SOD mimic behaviour was actively sought for clinical and mechanistic purposes under a nonenzymatic system (NBT/NADH/PMS), and was found to have good antioxidant activity.

1. Introduction

Synthesis of molecules which can serve as a probe for nucleic acid structure or which can show nuclease property has gained attention of many researchers [1]. Transition metal complexes have been widely exploited for these purposes not only because of their unique spectral and electrochemical signatures but also due to the fact that by changing the ligand environment one can tune the DNA binding and cleaving ability of a metal complex. Even though interaction of DNA with complexes of many transition metal ions have been investigated [2–4]. Copper(II) complexes are the preferred molecules for bringing about DNA cleavage. This is due to the fact that copper(II) complexes can not only bring about oxidative cleavage of DNA but also hydrolytic, photolytic and electrolytic cleavage of DNA [5,6]. Copper(II) is known to play a significant role in naturally occurring biological systems as well as a pharmacological agent [7,8].

The term quinolones is commonly used for the quinolonecarboxylic acids or 4-quinolones, which are a group of synthetic antibacterial agents containing a 4-oxo-1,4-dihydroquinoline skeleton (Fig. 1a). Quinolone antibiotics are chelating agents for a variety of metal ions well known for their biological activity [9–13]. It was discovered that a fluorine atom at position 6 and a piperazine ring at position 7 greatly enhance the spectrum of activity. Fluoroquinolones are extremely useful for the treatment of a variety of infections [14–16]. Norfloxacin (Fig. 1b), a fluoroquinolone, is a 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA. Several human diseases such as cardiovascular and neurodegenerative disorders, cancer, inflammatory and ageing processes involve some form of oxidation at the subcellular level. Cells and tissues injury (oxidative stress) results from the imbalance between pro and antioxidant species [17]. Superoxide dismutase activity (SOD) in conjunction with catalase appears to be the most effective enzymatic defence against the toxicity of oxygen metabolism [18–20]. Among the known SOD enzymes, Cu_2Zn_2(SOD) is the most efficient catalytic species. It catalysed the disproportionation of the cytotoxic superoxide radical $O_2^{\cdot-}$, to oxygen and hydrogen peroxide, through one-electron redox cycle involving its copper centre.

It is known since three decades ago that cancer cells have less than normal SOD activity and the treatment with bovine native Cu–SOD decreased the growth of several solid tumours [18]. Furthermore, low molecular weight compounds with superoxide dismutase mimetic activity have potential use as antioxidant pharmaceuticals in the treatment or prevention of several diseases related with the overproduction of an undesired $O_2^{\cdot-}$. In particular, some copper complexes with SOD mimetic activity have

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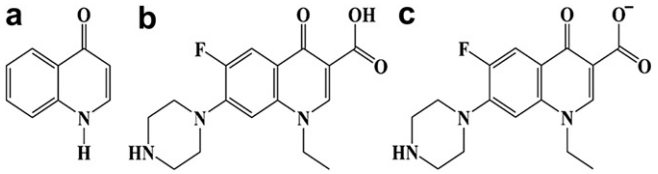


Fig 1. (a) Basic skeleton of quinolones; 4-oxo-1,4-dihydroquinoline. (b) 1-Ethyl-6-fluro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylic; norfloxacin (NFLH). (c) 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylate; deprotonated norfloxacin (NFL).

demonstrated to possess antiinflammatory activity, anticarcinogenic and antimutagenic effects [19,20]. These facts encouraged the investigation of model complexes, especially copper(II) with SOD mimetic activity [21,22] and have motivated us to include the study of low molecular weight copper complexes with antioxidant compounds in our research on metal coordination compounds with biological relevant ligands.

Herein, we have studied the biological properties of terpyridines–norfloxacin–copper(II) complexes including DNA interaction and enzymatic activity.

2. Experiments

2.1. Reagent

All reagents, chemicals and solvents used were of analytical reagent grade; double-distilled water was used throughout. Norfloxacin was generously supplied by Bayer AG (Wuppertal, Germany). Cupric chloride dihydrate was purchased from E. Merck Ltd, Mumbai (India). 2-Acetyl pyridine, *p*-chloro benzaldehyde, *p*-methyl benzaldehyde, *p*-bromo benzaldehyde, *p*-fluoro benzaldehyde, pyridine-2-carbaldehyde, thiophene-2-carbaldehyde, *p*-benzyloxy benzaldehyde, nicotinamide adenine dinucleotide reduced (NADH), nitro blue tetrazolium (NBT) and phenazine methosulphate (PMS) were purchased from Loba Chemie PVT. LTD. (India). Ethidium bromide, bromophenol blue, agarose and Luria Broth (LB) were purchased from Himedia (India).

2.2. Physical measurement

Microanalyses (C, H, and N) were done using a 240 Perkin Elmer elemental analyzer. Metal content of the complexes was determined by EDTA titration [23] after decomposing the organic matter with a mixture of $HClO_4$, H_2SO_4, and HNO_3 (1:1.5:2.5). Room temperature magnetic measurement for the complexes was made using Gouy magnetic balance. The Gouy tube was calibrated using mercury(II)tetrathiocyanatocobaltate(II), a calibrant ($\chi_g = 16.44 \times 10^{-6}$ cgs units at 20 °C). Electronic spectra were recorded on a UV-160A UV–Vis spectrophotometer, Shimadzu (Japan). Infrared spectra were recorded on an FT–IR Shimadzu spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. The minimum inhibitory concentration (MIC) study was carried out by means of laminar air flow cabinet Toshiba, Delhi (India). The LC–MS were recorded using Thermo mass spectrophotometer (USA). Photoquantization of the gel after electrophoresis was done using AlphaDigiDoc™ RT, version 4.0.0 PC-Image software (California, USA).

2.3. Synthesis of ligands

All tridentate ligands were synthesized similarly by following literature procedure [24]. 2-Acetyl pyridine (20.0 mmol) was added to an ethanolic solution of various aldehydes (10.0 mmol in 70 mL EtOH). KOH pellets (26 mmol) and aqueous NH_3 (25%, 0.425 mol) were added to the solution and was stirred at room temperature for 8 h. An off-white solid formed which was collected by filtration, followed by washings with H_2O (3 × 10 mL) and EtOH (2 × 5 mL). Crystallization from $CHCl_3$–MeOH system gives a white crystalline solid. The proposed reaction is shown in Scheme 1.

2.4. Synthesis of complexes

2.4.1. [Cu(NFL)(L^1)Cl]

Methanolic solution of $CuCl_2 \cdot 2H_2O$ (1.5 mmol) was added to a methanolic solution of 4′-(4-chlorophenyl)-2,2′:6′,2″-terpyridine (L^1) (1.5 mmol), followed by the addition of a previously prepared methanolic solution of norfloxacin (1.5 mmol) in presence of CH_3ONa (1.5 mmol). The pH of the reaction mixture was adjusted to ~6.8. The resulting solution was refluxed for 2 h on a water bath, followed by concentrating to half of its volume. A fine, green amorphous product obtained was washed with ether/hexane and dried in a vacuum desiccator. (Scheme 2) Yield: 65.2%, m.p.: >300 °C, μ_{eff}: 1.90 B.M. Anal. Calcd. for: $C_{38}H_{31}Cl_2FCuN_6O_3$ (761.13): C, 58.39; H, 4.11; N, 11.04; Cu, 8.35%. Found: C, 58.29; H, 4.02; N, 10.95; Cu, 8.29%.

2.4.2. [Cu(NFL)(L^2)Cl]

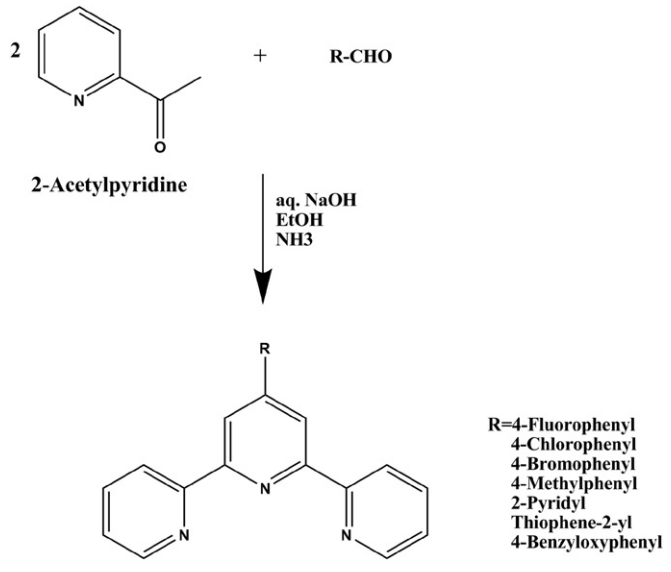
It was synthesized using 4′-(4-bromophenyl)-2,2′:6′,2″-terpyridine (L^2) (1.5 mmol). Yield: 64.5%, m.p.: 289 °C, μ_{eff}: 1.89 B.M. Anal. Calcd. for: $C_{37}H_{31}BrClFCuN_6O_3$ (805.58): C, 55.16; H, 3.88; N, 10.43; Cu, 7.89%. Found: C, 55.05; H, 3.78; N, 10.31; Cu, 7.80%.

2.4.3. [Cu(NFL)(L^3)Cl]

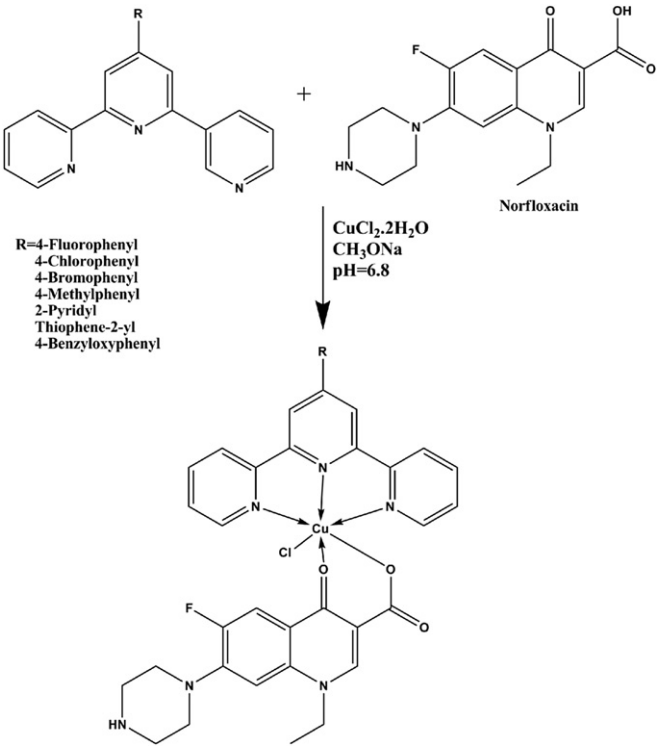
It was synthesized using 4′-(4-fluorophenyl)-2,2′:6′,2″-terpyridine (L^3) (1.5 mmol). Yield: 66.7%, m.p.: 279 °C, μ_{eff}: 1.92 B.M. Anal. Calcd. for: $C_{37}H_{31}ClF_2CuN_6O_3$ (744.68): C, 59.68; H, 4.20; N, 11.29; Cu, 8.53%. Found: C, 59.58; H, 4.09; N, 11.18; Cu, 8.45%.

2.4.4. [Cu(NFL)(L^4)Cl]

It was synthesized using 4′-(4-methylphenyl)-2,2′:6′,2″-terpyridine (L^4) (1.5 mmol). Yield: 68.3%, m.p.: >300 °C, μ_{eff}: 1.95 B.M. Anal. Calcd. for: $C_{38}H_{34}ClFCuN_6O_3$ (740.71): C, 61.62; H, 4.63; N, 11.35; Cu, 8.58%. Found: C, 61.50; H, 4.52; N, 11.46; Cu, 8.49%.



Scheme 1. General synthesis of Ligand.



Scheme 2. General synthesis of complex.

2.4.5. [Cu(NFL)(L^5)Cl]

It was synthesized using 4′-(2-pyridyl)-2,2′:6′,2″-terpyridine (L^5) (1.5 mmol). Yield: 62.7%, m.p.: 285 °C, μ_{eff}: 1.97 B.M. Anal. Calcd. for: $C_{36}H_{31}ClFCuN_7O_3$ (727.67): C, 59.42; H, 4.29; N, 13.47; Cu, 8.73%. Found: C, 59.31; H, 4.36; N, 13.56; Cu, 8.63%.

2.4.6. [Cu(NFL)(L^6)Cl]

It was synthesized using 4′-(thiophen-2-yl)-2,2′:6′,2″-terpyridine (L^6) (1.5 mmol). Yield: 63.1%, m.p.: >300 °C, μ_{eff}: 1.87 B.M. Anal. Calcd. for: $C_{35}H_{30}ClFCuN_6O_3S$ (732.71): C, 57.37; H, 4.13; N, 11.47; Cu, 8.67%. Found: C, 57.44; H, 4.20; N, 11.38; Cu, 8.58%.

2.4.7. [Cu(NFL)(L^7)Cl]

It was synthesized using 4′-(4-benzyloxyphenyl)-2,2′:6′,2″-terpyridine (L^7) (1.5 mmol). Yield: 64.2%, m.p.: >300 °C, μ_{eff}: 1.93 B.M. Anal. Calcd. for: $C_{44}H_{38}ClFCuN_6O_4$ (832.81), C, 63.46; H, 4.60; N, 10.09; Cu, 7.63%. Found: C, 63.38; H, 4.70; N, 9.98; Cu, 7.54%.

2.5. Antibacterial activity

The complexes were screened for their *in vitro* antibacterial activity against two Gram$^{(+ve)}$ i.e., *Staphylococcus aureus*, *Bacillus subtilis*, and three Gram$^{(-ve)}$ i.e., *Serratia marcescens*, *Pseudomonas aeruginosa*, and *Escherichia coli* species by minimum inhibitory concentration (MIC) using Broth dilution method. Two percent of sterile LB was used as a medium which consists of 1% w/v tryptone, 0.5% w/v NaCl, and 0.5% w/v yeast extract. All the cultures were incubated at 37 °C. Control tests with no active ingredients and the solvent alone were also performed. Preculture of the bacteria used were grown in LB overnight at an optimum temperature for each species. Bacterial growths were measured by the turbidity of the culture after incubation for 18 h. If a particular concentration of compound inhibits the bacterial growth, half the concentration of the compound was tried. The procedure was continued to a concentration at which bacteria grow normally. The lowest concentration that inhibits the bacterial growth was considered as the MIC value. Surroundings were maintained sterile throughout.

The bactericidal action of all compounds was evaluated against same microorganisms. The inoculum was prepared by diluting an overnight culture grown in Luria Broth to obtain 10^6 viable bacteria/mL. Bacteria were exposed to various concentrations of compounds. Compound-free control tubes were included in each run. The final volume was 1 mL. Cultures were incubated at 37 °C for 2 h. The 100 μL bacterial culture from each dilution was taken and spread over previously prepared agar plate. The plates were incubated for 24 h and colony counts were performed on plates yielding 30–300 colonies.

2.6. DNA interaction activity

2.6.1. Absorption titration

Binding of Herring Sperm DNA via intercalative mode usually results in hypochromism and bathochromism [25,26] because the intercalation mode involves a strong stacking interaction between an aromatic chromophore and the DNA base pair [27]. After addition of an equivalent amount of DNA to the reference cell, it was kept for 10 min incubation at room temperature followed by absorption measurement around an appropriate absorbance peak. This was specifically done to enable direct comparison between the assays which was required to interpret the results obtained. The intrinsic binding constant, K_b for each complex was determined by making it subject to the following equation [28]:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

where, [DNA] is the concentration of Herring Sperm DNA in base pairs, ε_a corresponds to the apparent extinction coefficient obtained by calculating A_{obsd}/[complex], ε_f corresponds to the extinction coefficient of the complex in its free form and ε_b refers to the extinction coefficient of the complex in the fully bound form. The data were fitted to the above equation to obtain straight line with a slope value corresponding to the term $1/(\varepsilon_a - \varepsilon_f)$ and the *y*-intercept to $1/K_b(\varepsilon_b - \varepsilon_f)$. The K_b was determined from the ratio of slope to the *y*-intercept.

2.6.2. DNA binding study by hydrodynamic volume measurement

Ubbelohde viscometer immersed in a thermostatic bath maintained at 27 ± 0.1 °C was used to measure the change in hydrodynamic volume with change in complex concentration. Digital stopwatch with least count of 0.01 s was engaged for flow times measurement with accuracy of ±0.1 s. Plot of $(\eta/\eta_0)^{1/3}$ *versus* [complex]/[DNA] is used to study the behavior of binding, where η is the viscosity of DNA in presence of complex and η_0 is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA-containing solutions (t) corrected for that of the buffer alone (t_0), $\eta = (t - t_0)$ [29].

2.6.3. DNA cleavage study by gel electrophoresis

Gel electrophoresis of plasmid DNA (pUC19 DNA) was carried out in Tris-acetate-EDTA (TAE) buffer (0.04 M TAE, pH 8, 0.001 M EDTA). 15 μL reaction mixture containing plasmid DNA in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and 200 μM complex. Reactions were allowed to proceed for 24 h at 37 °C. All reactions were quenched by addition of 5 μL loading buffer (0.25% bromophenol blue, 40% sucrose, 0.25% xylene cyanole, and 200 mM EDTA). The aliquots were loaded directly on to 1% agarose gel and electrophoresed at 50 V in 1X TAE buffer. Gel was stained with 0.5 μg/mL EtBr and was photographed on a UV illuminator. The percentage of

Table 1
Change in IR bands for interaction of norfloxacin with Cu(II) in addition to terpyridines (4000–400 cm^{-1}).

Compounds	ν(C=O) cm^{-1} pyridine	$\nu(COO)_{asym}$ cm^{-1}	$\nu(COO)_{sym}$ cm^{-1}	$\Delta\nu$ cm^{-1}	ν(M–N) cm^{-1}	ν(M–O) cm^{-1}
Norfloxacin	1730	1642	1336	306	–	–
Complex-**1**	1620	1568	1368	200	534	516
Complex-**2**	1615	1588	1385	203	543	507
Complex-**3**	1630	1570	1365	205	539	512
Complex-**4**	1622	1577	1369	208	536	510
Complex-**5**	1628	1591	1381	210	541	518
Complex-**6**	1625	1572	1360	212	546	520
Complex-**7**	1635	1594	1387	207	549	523

each form of DNA was quantities using AlphaDigiDoc™ RT. Version V.4.0.0 PC–Image software.

2.7. Enzymatic behavior

NBT/NADH/PMS system was used to study SOD-like behavior of the complexes. The superoxide radial produced by 79 μM NADH, 30 μM PMS in phosphate buffer (pH = 7.8) was responsible for reduction of 75 μM NBT in system, and 0.5–3.0 μM tested compound are responsible for retardation in the reduction rate of NBT which was determined spectrophotometrically by monitoring the concentration of blue formazan form which absorbs at 560 nm. All measurements were carried out at room temperature. The % inhibition (η) of NBT reduction was calculated using following equation [30]:

$$(\%\ \text{Inhibition of NBT reduction}) = (1 - k'/k) \times 100$$

where, $k'$and k represent the slopes of the straight line of absorbance values as a function of time in presence and in absence of SOD mimic or a model compound, respectively. IC_{50} value of the complex was determined by plotting the graph of percentage inhibition of NBT reduction against increase in the concentration of complex. Concentration of the complex which causes 50% inhibition of NBT reduction is reported as IC_{50}.

3. Result and discussion

3.1. Magnetic and electronic behaviour

The magnetic moments of copper(II) in any of its geometry lies around 1.8 B.M. which is very close to spin-only value i.e. 1.73 B.M. The found values in our case lie in the range, 1.87–1.96 B.M. These values are typical for mononuclear copper(II) compounds having d^9-electronic configuration. The observed magnetic moments of all the complexes correspond to typical high-spin octahedral complexes. However, the values are slightly higher than the expected spin-only values due to spin orbit coupling contribution [31]. Visible emission spectra of the copper(II) complexes, i.e. d^9 system, were recorded in DMSO. Complexes exhibited one broad band along with a shoulder at ~14,000 cm^{-1} and ~11,000 cm^{-1} respectively, attributed to the d–d transition for Cu(II) atom in a distorted octahedral geometry [32].

3.2. IR spectra

Determination of the coordinating atoms was done on the basis of the comparison of IR spectra of norfloxacin and of the complexes. Significant wave numbers are given in Table 1. For norfloxacin, the ν(C=O) stretching vibration band appears at 1730 cm^{-1} whereas for complexes it appears in the range 1615–1635 cm^{-1}. This shift in band towards lower energy suggests that coordination occurs through the pyridone oxygen atom. In case of norfloxacin, strong absorption bands at 1642 cm^{-1} and 1336 cm^{-1} could be assigned for $\nu(COO)_{assy}$ and $\nu(COO)_{sym}$ vibration respectively. While for metal complexes these bands were observed in the range 1568–1594 and

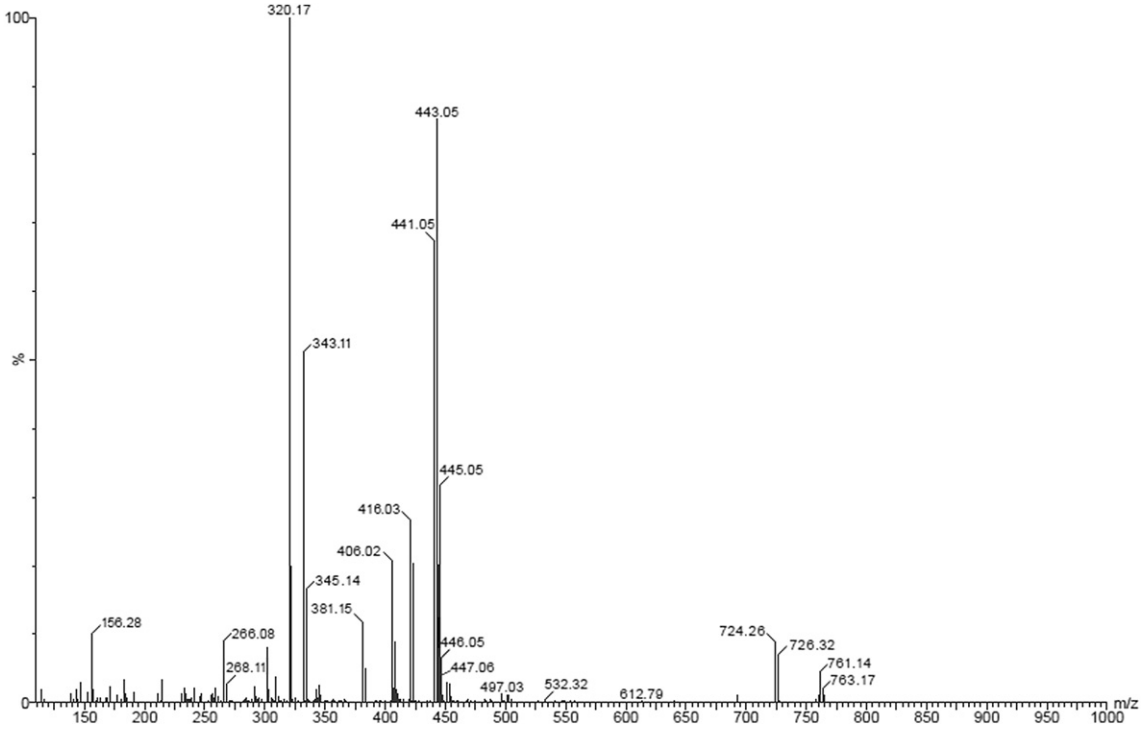
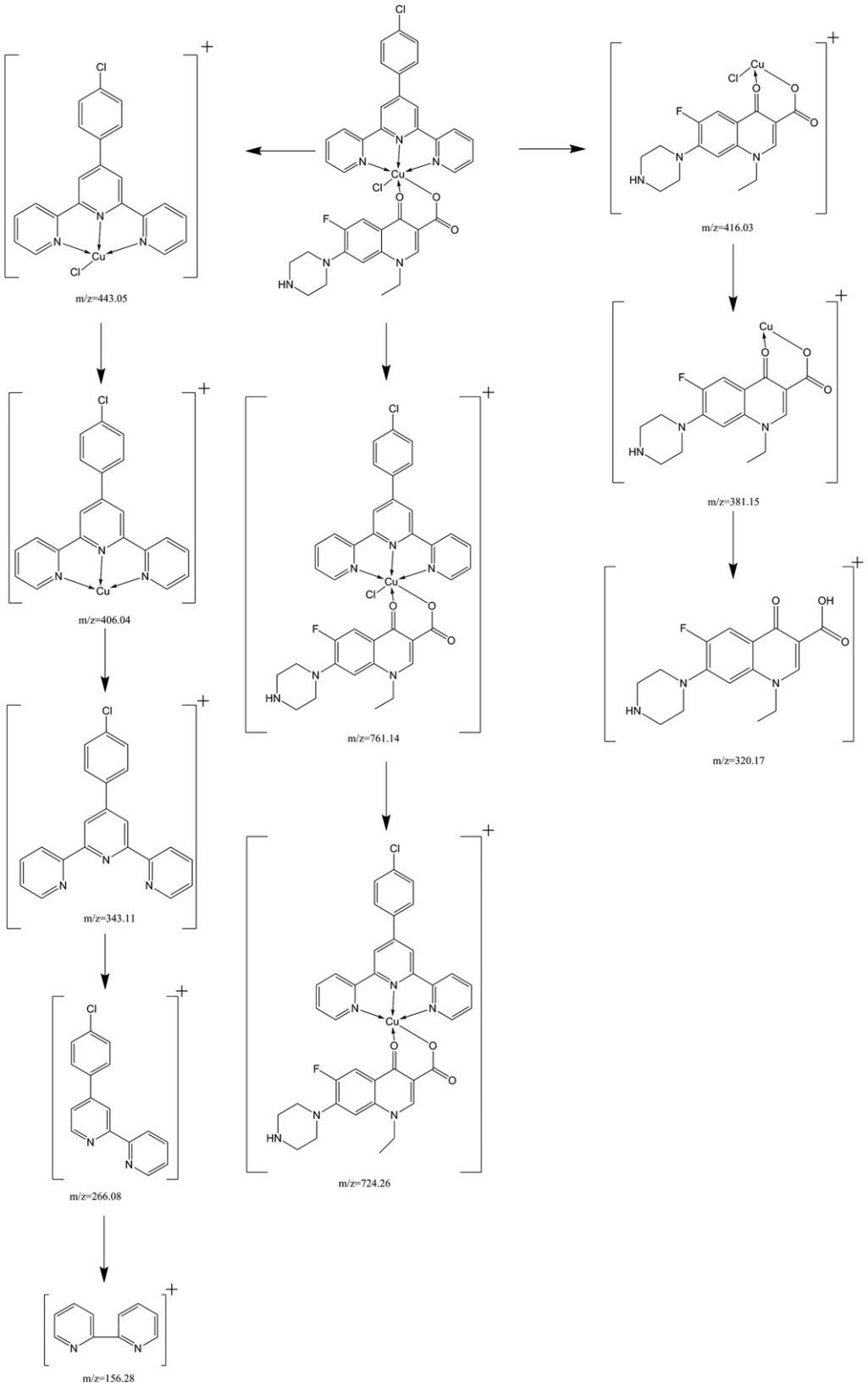


Fig. 2. LC-mass spectrum of complex **1**, that is [Cu(NFL)(L^1)Cl].



Scheme 3. Fragmentation pattern of [Cu(NFL)(L^1)Cl].

Table 2
Bacteriostatic concentration of $CuCl_2 \cdot 2H_2O$, NFLH and complexes by Broth dilution in terms of MIC (μmol L^{-1}).

Compounds	Gram (+ve)		Gram (−ve)		
	S. aureus	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
$CuCl_2 \cdot 2H_2O$	2698.0	2815.0	2756.0	2404.0	3402.0
Norfloxacin	2.4	2.5	3.9	3.7	2.7
1	0.65	0.7	1.25	1.1	0.9
2	1.0	1.05	1.7	1.6	1.2
3	0.5	0.6	1.0	0.9	0.7
4	1.45	1.5	2.6	2.4	1.9
5	2.5	2.6	4.2	3.9	2.9
6	1.9	2.0	3.3	3.15	2.3
7	2.75	2.8	4.5	4.1	3.2

1360–1387 cm^{-1}. To determine coordination mode of ligands, the difference $\Delta = \nu(COO)_{assy} - \nu(COO)_{sym}$ is very useful. The Δ values are greater than 200 cm^{-1}, which indicate the monodentate coordination mode of carboxylato group of the ligands [33–37]. These data are further supported by ν(M–O), which appears in the range 507–523 cm^{-1} for complexes. In investigated complexes the ν(C=N) band of terpyridine appears at 1580 cm^{-1}. This band shift to higher frequency at ~1630 cm^{-1} in complexes, which indicates the tridentate N–N coordination of the ligand [38,39]. The N → M bonding was supported by ν(M–N) band at ~534–549 cm^{-1} for complexes [40].

3.3. Mass spectra

The mass spectrum of complex **1** showed molecular ion peak at $m/z = 761.14$. The doublet observed at $m/z = 724.26$ and 726.32 was due to the presence of one chlorine atom. In case of copper–terpyridine moiety, the doublet observed at $m/z = 443.05$ and 445.05 confirms the presence of one chlorine atom. The doublet was observed at m/z = 406.02 and 408.02 corresponding to the copper–terpyridine moiety. The doublet observed at $m/z = 416.03$ and 418.03 also confirms the presence of one chlorine atom in case of copper–norfloxacin moiety. The peak at m/z = 381.15 was observed due to loss of chlorine atom from the copper–norfloxacin fragment. The doublet was observed at m/z = 343.11 and 345.14 corresponding to presence of one chlorine atom in the terpyridine moiety. Other peaks at $m/z = 266.08$ and 156.28 were observed due to fragmentation of terpyridine moiety. The peak was observed at m/z = 320.17 corresponding to norfloxacin. Fig. 2 represents the mass spectrum of Complex **1**. The proposed fragmentation pattern of complex **1** is shown in Scheme 3.

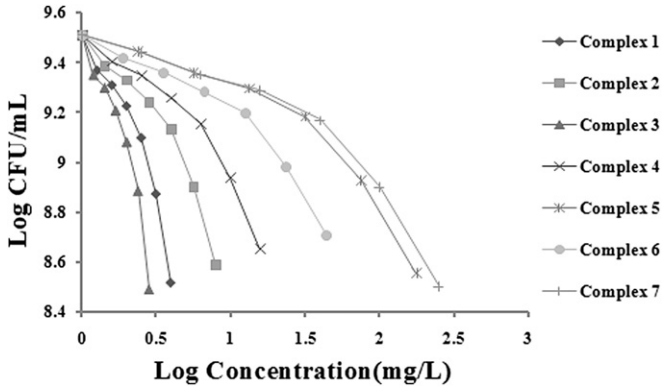


Fig. 3. Relationship between concentration and bactericidal activity of all complexes against *S. aureus*.

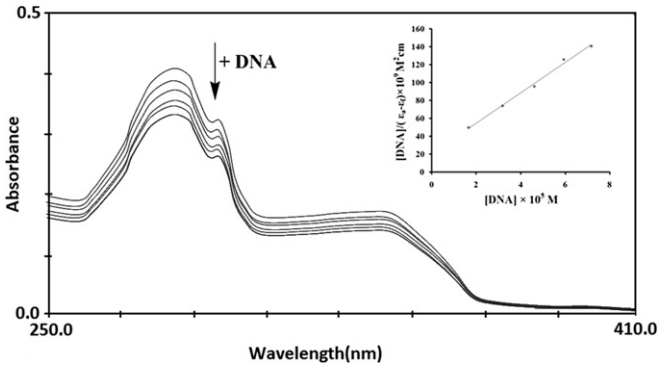


Fig. 4. Electronic absorption spectra of **1** in phosphate buffer (Na_2HPO_4–NaH_2PO_4, pH 7.2) in the absence and presence of increasing amount of DNA. The [complex] = 10 μM; [DNA] = 0–150 μM. The incubation period is 10 min at 37 °C. Inset: plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA]. Arrow shows the absorbance change upon increasing DNA concentrations.

3.4. Antibacterial activity

Table 2 shows *in vitro* antibacterial activity data of the cupric chloride dihydrate, norfloxacin and synthesized complexes (**1**–**7**). It is clear from the data that complexes **1**, **2**, **3**, **4**, **6** are active compare to NFLH for all the bacterial species employed. Complexes **5** and **7** are less active than NFLH against all the species. Out of all the active complexes, complex **3** is the most active one. The observed higher antimicrobial activities of the complexes can be explained on the basis of Tweedy's chelation theory [41]. The lipid membrane that surrounds the cell favours the passage of only lipid soluble materials due to which lipophilicity is an important factor which controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with the donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and hence enhances the liposolubility of the complexes. This increased liposolubility enhances the penetration of the complexes into the lipid membrane; the lipophilic group to drive the compound through the semipermeable membrane of the cell; and blocks the metal binding sites in the enzymes of the microorganisms.

In addition, our study regarding bactericidal activity in terms of CFU/mL of above metal complexes against same microorganisms (three $Gram^{(-ve)}$ and two $Gram^{(+ve)}$) revealed decrease in number of colonies with increase in the concentration of compounds. The results are shown in Fig. 3 for all the complexes against *S. aureus*. The number of colonies counted in this technique was 30–300. From the minimum inhibitory concentration (MIC) values and colony forming units (CFU), it is found that complex **3** is more potent against all the five microorganisms.

Table 3
The binding constants (K_b) of complexes with DNA in phosphate buffer pH 7.2.

Complexes	K_b (M^{-1})
[Cu(NFL)(L^1)Cl]	8.31×10^4
[Cu(NFL)(L^2)Cl]	6.63×10^4
[Cu(NFL)(L^3)Cl]	9.60×10^4
[Cu(NFL)(L^4)Cl]	4.12×10^4
[Cu(NFL)(L^5)Cl]	8.81×10^3
[Cu(NFL)(L^6)Cl]	2.40×10^4
[Cu(NFL)(L^7)Cl]	7.96×10^3

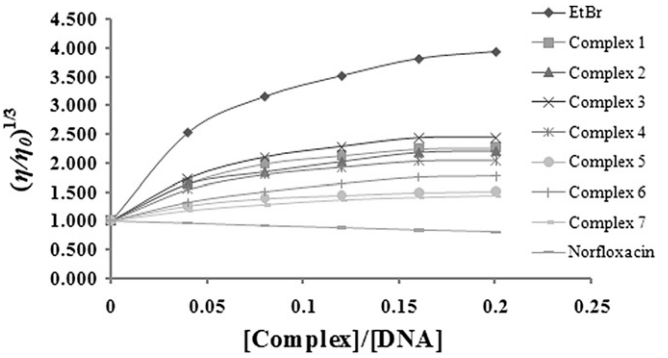


Fig. 5. Effect on relative viscosity of DNA under the influence of increasing amount of complexes at 27 ± 0.1 °C in phosphate buffer (Na_2HPO_4–NaH_2PO_4, pH 7.2).

3.5. DNA interaction study

3.5.1. Absorption titration experiment

DNA can provide three distinct binding sites for quinolone metal complexes, namely, groove, phosphate group and intercalation [42]. This behaviour is of great importance with respect to the relevant biological role of quinolone antibiotics in the body [43]. An absorption spectrum of complexes with Herring Sperm DNA was recorded for a constant concentration of complexes with varying concentration of DNA to obtain different DNA:complex mixing ratio. A representative titration curve is shown in Fig. 4. The changes observed in the UV spectra of the complexes after mixing it with DNA (either the increase in intensity or the shift in the wavelength) indicate that the interaction of complexes with DNA takes place by a direct formation of a new complex with double-helical DNA [44]. The extent of the binding strength of complexes was quantitatively determined by calculating intrinsic binding constant (K_b) of the complexes by monitoring the change in absorbance at various concentrations of DNA. From the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *versus* [DNA] (inset, Fig. 4), the K_b values of complexes were determined and were found in the range 7.96×10^3–9.60×10^4 M^{-1} (Table 3). The K_b value and red shift clearly indicates that the synthesized complexes bind to DNA via intercalation mode but the values are much lower compared with ethidium bromide, a classical intercalator [45].

3.5.2. DNA binding study by hydrodynamic volume measurement

Viscosity measurement can be regarded as a reliable tool in the absence of crystallographic and NMR data [46]. The intercalation of a molecule into DNA could result in lengthening, unwinding and stiffening of the helix and is usually accompany by increase in solution viscosity [47,48]. In this case, an increase in viscosity was observed hence the complexes were bound to DNA via an intercalation mode and out of all the complexes, complex **3** interacted more strongly compare to the others (Fig. 5).

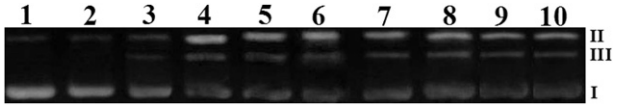


Fig. 6. Separation of cleaved pUC19 DNA (150 μg/mL) with series of copper(II) complexes (200 μmol L^{-1}) using 1% agarose gel stained with 0.5 μg/mL ethidium bromide. Interaction between DNA and complexes was allowed for 24 h at 37 °C in TE buffer (pH 8) with a final volume of 15 μL: Lane 1, DNA control; Lane 2, $CuCl_2 \cdot 2H_2O$; Lane 3, norfloxacin; Lane 4, [Cu(NFL)(L^1)Cl]; Lane 5, [Cu(NFL)(L^2)Cl]; Lane 6, [Cu(NFL)(L^3)Cl]; Lane 7, [Cu(NFL)(L^4)Cl]; Lane 8[Cu(NFL)(L^5)Cl]; Lane 9[Cu(NFL)(L^6)Cl]; Lane 10[Cu(NFL)(L^7)Cl].

Table 4
Experimental values of IC_{50} obtain from nonenzymatic SOD-like activities of synthesized complexes.

Complexes	IC_{50} (μM)
[Cu(NFL)(L^1)Cl]	0.591
[Cu(NFL)(L^2)Cl]	0.675
[Cu(NFL)(L^3)Cl]	0.552
[Cu(NFL)(L^4)Cl]	0.822
[Cu(NFL)(L^5)Cl]	1.244
[Cu(NFL)(L^6)Cl]	1.012
[Cu(NFL)(L^7)Cl]	1.276

3.5.3. DNA cleavage study by gel electrophoresis

DNA cleavage accelerated by transition metal complexes is the centre of interest [49]. Fig. 6 shows the electrophoretic separation of pUC19 DNA reacted with complexes under aerobic conditions. When the plasmid DNA was subjected to electrophoresis upon reacting with complexes, the fastest migration was observed for the supercoiled (SC) form (form I), the slowest moving open circular (OC) form (form II) produced upon relaxing SC and the intermediate moving linear (LC) form (form III), generated upon the cleavage of circular form. Data for the cleavage study are presented in Table 5. The difference in the DNA cleavage efficiency of complexes was due to the difference in the binding affinity of the complexes to DNA.

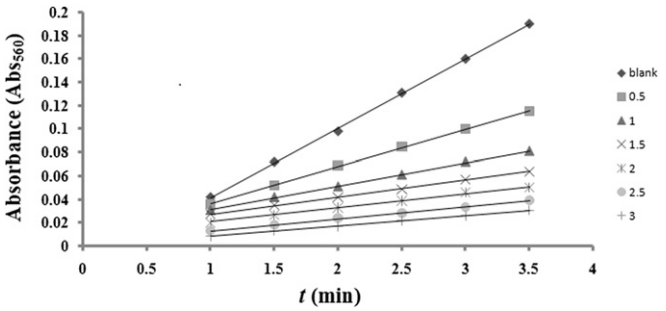


Fig. 7. Absorbance (Abs_{560}) as a function of time (t) plotted for varying concentration of complex **1** from 0.5 μM–3 μM.

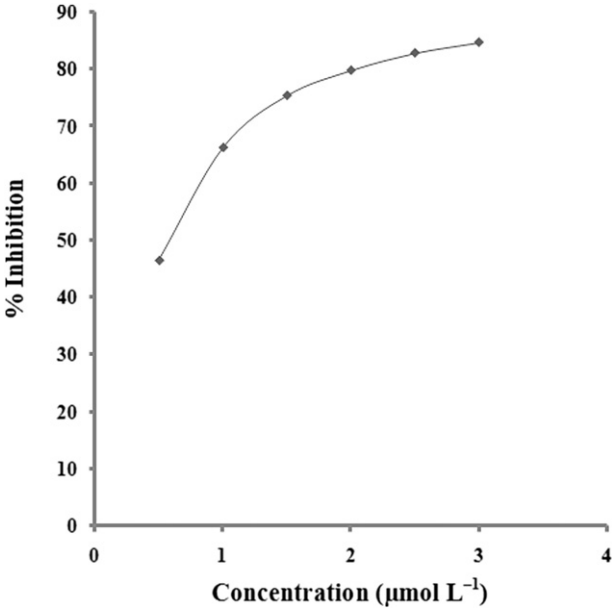


Fig. 8. Plot of percentage of inhibiting NBT reduction with an increase in the concentration of complex **1**.

Table 5
Complex mediated DNA cleavage data by gel electrophoresis.

Lane No.	Compound	Form I (SC)	Form II (OC)	Form III (LC)
1	Control	91	9	–
2	$CuCl_2 \cdot 2H_2O$	87	13	–
3	Norfloxacin	63	22	15
4	[Cu(NFL)(L^1)Cl]	27	56	17
5	[Cu(NFL)(L^2)Cl]	25	59	16
6	[Cu(NFL)(L^3)Cl]	20	62	18
7	[Cu(NFL)(L^4)Cl]	28	53	19
8	[Cu(NFL)(L^5)Cl]	33	52	15
9	[Cu(NFL)(L^6)Cl]	30	51	19
10	[Cu(NFL)(L^7)Cl]	35	49	16

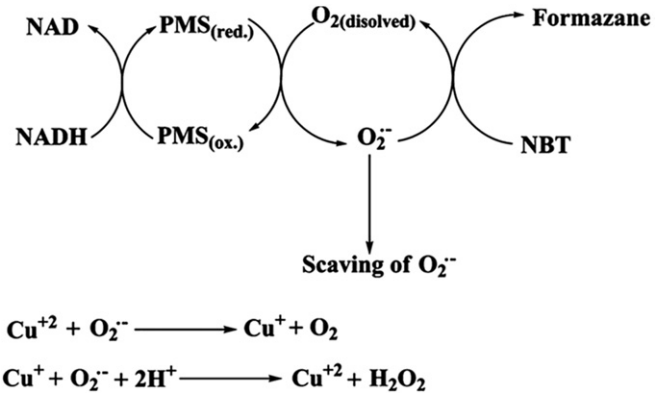


Fig. 9. Mechanism for SOD-like activity performed using the NBT/NADH/PMS system.

3.6. Enzymatic behavior

The NADH/PMS/NBT system was used to generate the superoxide radical artificially in order to check SOD-like behavior of the complexes. The percentage inhibition of formazan formation at various concentrations of the complexes as a function of time was determined by measuring the absorbance at 560 nm and this was plotted to give a straight line obeying the equation $Y = mX + C$ (Fig. 7). With an increase in the concentration of tested complexes a decrease in the slope (m) was observed. The percentage inhibition of the reduction rate of nitro blue tetrazolium (NBT) was plotted against the concentration of the complex to obtain the IC_{50} value (Fig. 8). The compounds exhibited SOD-like activity at biological pH with their IC_{50} values ranging from 0.552 to 1.276 μM (Table 4). The best IC_{50} value among the synthesised complexes was observed for complex **3**. The proposed mechanism for generation of ROS and its dismutation is shown in Fig. 9 [50]. Fee and Huheey proposed rapid interconversion between Cu(II) and Cu(I) via electron transfer between copper and reactive oxygen radical anion following the principle of electroneutrality [51].

4. Conclusions

Herein this work, we have synthesized seven Cu(II) metallointercalators with various terpyridines and norfloxacin. While comparing the data of MIC for complexes and drug, complexes **1**, **2**, **3**, **4** and **6** showed good results for all microorganisms, among which complex **3** fall out with highest potency. Reason for increase in potency of drug is its coordination with metal ion. From the viscosity data of complexes and classical intercalator ethidium bromide; it is clear that all complex show classical intercalative mode of binding, where complex **3** binds more strongly than rest of the complexes. The electronic absorption data are in good accordance with viscosity study. The DNA cleavage study of pUC19 shows that all complexes have high cleavage ability than metal salt and drug. Upon determination of antioxidant activity in NBT/NADH/PMS system, complex **3** shows the highest scavenging ability for oxygen radical. The preliminary studies encourage for carrying out further in vivo experiments and seeming helpful in generating database for preparation of effective DNA probe.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Appendix. Supplementary Data

Supplementary data related to this article can be found online at doi:10.1016/j.jorganchem.2011.11.022.

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