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Synthesis, Characterization and Biological Activities of Ciprofloxacin Drug Based Metal Complexes

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Abstract

The interaction of small molecules with DNA has attracted a great deal of attention. Mixed ligand copper(II) complexes of type [Cu(cpf)(L^n)Cl] [cpf = ciprofloxacin, L^n = phenanthroline derivatives] were synthesized and characterized by elemental analysis, reflectance, IR and mass spectra. Viscosity measurements, absorption titration and DNA melting temperature studies were employed to determine the mode of binding of complexes with DNA. DNA cleavage study showed better cleaving ability of the complexes compare to metal salts and standard drug. The SOD mimic study showed IC_{50} value of complexes in the range of 0.95 to 1.75 μM. Antibacterial activity was assayed against selective $Gram^{(-ve)}$ and $Gram^{(+ve)}$ microorganisms.

Keywords: Neucleolytic activity, Calf thymus DNA, Phenanthroline, Absorption titration, DNA Melting temperature

1. Introduction

Copper is an essential micronutrient and found in several proteins and enzymes. Numerous copper compounds are able to act as antioxidant, antimicrobial, antiparasitic, anti-inflammatory, anticonvulsant and antitumoral agents. Copper complexes with ligands containing nitrogenated aromatic rings have deserved a great interest since the complex of 1,10-phenanthroline proved its ability to break DNA chains.[1,2] Cells and tissues injury (oxidative stress) results from the imbalance between pro and antioxidant species.[3] Superoxide dismutase activity (SOD) in conjunction with catalase appears to be the most effective enzymatic defence system against the toxicity of oxygen metabolism.[4,5] Among the known SOD enzymes, CuZn(SOD) is the most efficient catalytic species.

Recently, there has been considerable interest in the DNA binding properties of transition metal complexes with small molecules on a molecules level.[6] The design of complexes that bind and react with DNA becomes important as we begin to delineate the expression of genetic information on a molecular level. Recently, the complexes of vanadium(IV), copper(II), magnesium(II), uranium(VI), manganese(II), iron(III), cobalt(II), nickel(II), molybdenum(II) and europium(III) with fluoroquinolones have been synthesized and explored for their biological activities, because of its biological relevance.[7–13] Targeting above facts and in continuation to our earlier work,[14] herein, the interaction of Cu(II) with the ciprofloxacin in the presence of a nitrogen donor heterocyclic ligand (phenanthrolines) has been studied in an attempt to examine their biological behavior.

2. Experiments

2. 1. Materials-Instrumentation

All the chemicals and solvents used were of analytical grade. Ciprofloxacin hydrochloride was purchased from Bayer AG (Wuppertal, Germany). Cupric chloride dihydrate, 3-chlorobenzaldehyde, 3-bromobenzaldehyde, 3-benzyloxybenzaldehyde, 4-benzyloxybenzaldehyde, 9-anthraldehyde, pyridine-2-carbaldehyde, pyridine-3-carbaldehyde, thiophene-2-carbaldehyde, benzaldehyde, acetic acid and EDTA were purchased from Sd fine chemicals (India). Ethidium bromide and Luria Broth were purchased from Himedia (India). CT DNA was purchased from Sigma Chemicals Co. (India).

Metal contents of the complexes were analyzed gravimetrically and volumetrically,[15] after decompo-

sing the organic matter with acid mixture ($HClO_4$, H_2SO_4 and HNO_3). C, H and N elemental analyses were performed on a Perkin-Elmer 240 elemental analyzer. Magnetic moments were measured by Gouy's method using mercury tetrathiocyanatocobaltate(II) as the calibrant ($\chi_g = 16.44 \times 10^{-6}$ cgs units at 20 °C), on Citizen Balance. The diamagnetic correction was made using Pascal's constant. IR spectra were recorded on FT-IR Shimadzu spectrophotometer with sample prepared as KBr pellets in the range 4000–400 cm^{-1}. The reflectance spectra were recorded on a LAMBDA 19 UV/Vis/NIR spectrophotometer, Perkin Elmer (USA). The FAB mass spectra were recorded on a Jeol SX 120/Da-600 mass spectrometer/ Data system using Argon/Xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature.

2. 2. Synthesis of Ligands

2-(3-Bromophenyl)-1H-imidazo[4,5-f][1,10]phenanthroline (L^1)

A mixture of 3-bromobenzaldehyde (3.5 mmol), 1,10-phenanthroline-5,6-dione (3.5 mmol, 0.530 g), ammonium acetate (50 mmol), and glacial acetic acid (10 mL) were refluxed for about 2 h, then cooled to room temperature, and diluted with water (25 mL). Dropwise addition of concentrated aqueous ammonia (5 mL) till it gives yellow precipitates, followed by washing with water gave crude product. The crude products were purified by silica gel filtration (60–100 mesh, ethanol). The principal yellow band was collected.

Yield: 36%; m.p.: 181–183 °C; Anal. Calc. for $C_{19}H_{11}BrN_4$ (375.22 g/mol): Calc. (%):C, 60.82; H, 2.95; N, 14.93; Found (%): C, 60.72; H, 2.78; N, 14.83. ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm: 13.773 (s, 1H, NH), 9.055 (d, 2H, $H_{2',2''}$), 9.006 (d, 2H, $H_{4',4''}$), 8.314 (s, 1H, H_{Ph2}), 8.274 (d, 1H, H_{Ph6}), 7.841 (t, 2H, $H_{3',3''}$), 7.681 (t, 1H, H_{Ph5}), 7.592 (d, 1H, H_{Ph4}), ^{13}C NMR ($CDCl_3$, 100 MHz): 156.15 ($C_{6',6''}$), 155.27 (C_2), 148.53 ($C_{2',2''}$), 132.89 (C_{Ph3}), 132.66 (C_{Ph4}), 131.48 (C_{Ph1}), 131.34 ($C_{4,5}$), 130.16 (C_{Ph2}), 129.19 (C_{Ph5}), 126.54 (C_{Ph6}), 125.41 ($C_{5',5''}$), 124.91 ($C_{4',4''}$), 122.15 ($C_{3',3''}$).

2-(3-Chlorophenyl)-1H-imidazo[4,5-f][1,10]phenanthroline (L^2)

Similar procedure was followed by taking 3-chlorobezaldehyde.Yield: 32%; m.p.: 192–194 °C; Anal. Calc. for $C_{19}H_{11}ClN_4$ (330.77 g/mol): Calc. (%):C, 68.99; H, 3.35; N, 16.94; Found (%): C, 68.78; H, 3.28; N, 16.81. ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm: 13.770 (s, 1H, NH), 9.015 (d, 2H, $H_{2',2''}$), 8.862 (d, 2H, $H_{4',4''}$), 8.289 (s, 1H, H_{Ph2}), 8.226 (d, 1H, H_{Ph6}), 7.796 (t, 2H, $H_{3',3''}$), 7.616 (t, 1H, H_{Ph5}), 7.552 (d, 1H, H_{Ph4}), ^{13}C NMR ($CDCl_3$, 100 MHz): 154.16 ($C_{6,6'}$), 152.89 (C_2), 149.69 ($C_{2',2''}$), 139.61 (C_{Ph3}), 134.83 (C_{Ph4}), 132.30 (C_{ph1}), 130.27 ($C_{Ph4,5}$), 129.56 (C_{Ph2}), 128.89 (C_{Ph5}), 126.41 (C_{ph6}), 125.68 ($C_{Ph5,5''}$), 124.13 ($C_{4',4''}$), 121.55 ($C_{3',3''}$).

2-(4-(Benzyloxy)phenyl)-1H-imidazo[4,5-f][1,10]phenanthroline (L^3)

Similar procedure was followed by taking 4-benzyloxybezaldehyde. Yield: 38%; m.p.: 185–187 °C; Anal. Calc. for $C_{26}H_{18}N_4O$ (402.42 g/mol): Calc. (%):C, 77.59; H, 4.51; N, 13.92; Found (%): C, 77.42; H, 4.33; N, 13.81. ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm: 13.589 (s, 1H, NH), 9.030–9.019 (complex, 2H, $H_{2',2''}$), 8.904 (d, 2H, $H_{4',4''}$), 8.229 (d, 2H, $H_{Ph2,6}$), 7.854–7.789 (complex, 2H, $H_{3',3''}$), 7.515 (d, 2H, $H_{Bz2,6}$), 7.430 (t, 2H, $H_{Bz5,3}$), 7.362 (t, 1H, Bz_4), 7.266 (d, 2H, $Ph_{3,5}$), 5.221 (s, 2H, OCH_2), ^{13}C NMR ($CDCl_3$, 100 MHz): 159.03 (C_{Ph4}) 154.09 ($C_{6',6''}$), 153.14 (C_2), 149.16 ($C_{2',2''}$), 136.26 (C_{Bz1}), 130.01 ($C_{4,5}$), 131.23 ($C_{Ph2,6}$), 128.96 ($C_{Bz3,5}$), 127.66 (C_{Bz4}), 127.53 ($C_{Bz2,6}$), 126.54 ($C_{5',5''}$), 124.42 ($C_{4',4''}$), 121.20 ($C_{3',3''}$), 115.26 ($C_{Ph3,5}$), 113.44 (C_{Ph1}), 69.96 (CH_2).

2-(3-(Benzyloxy)phenyl)-1H-imidazo[4,5-f][1,10]phenanthroline (L^4)

Similar procedure was followed by taking 3-benzyloxybezaldehyde. Yield: 35%; m.p.: 188–190 °C; Anal. Calc. for $C_{26}H_{18}N_4O$ (402.42 g/mol): Calc. (%): C, 77.59; H, 4.51; N, 13.92; Found (%): C, 77.47; H, 4.39; N, 13.77. ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm: 13.563 (s, 1H, NH), 9.053–9.039 (complex, 2H, $H_{2',2''}$), 8.914 (d, 2H, $H_{4',4''}$), 8.248 (d, 2H, $Bz_{2,6}$), 7.895–7.801 (complex, 2H, $H_{3',3''}$), 7.516 (d, 1H, H_{Ph6}), 7.497–7.469 (complex, 5H, $H_{Ph2,5Bz3,4,5}$), 7.261 (d, 1H, H_{Ph4}), 5.216 (2H, OCH_2), ^{13}C NMR ($CDCl_3$, 100 MHz): 157.51 (C_{Ph3}), 154.13 ($C_{6',6''}$), 152.95 (C_2), 149.76 ($C_{2',2''}$), 136.88 ($C_{4,5}$), 132.51 (C_{Bz1}), 130.32 (C_{Ph5}), 128.71 ($C_{Bz3,5}$), 127.69 (C_{Bz4}), 127.18 ($C_{Bz2,6}$), 126.47 ($C_{5',5''}$), 125.25 (C_{Ph1}), 124.41($C_{4',4''}$), 121.55 ($C_{3',3''}$), 119.81 (C_{Ph6}), 114.20 (C_{Ph4}), 111.31 (C_{Ph2}), 70.81 (CH_2).

2-(Anthracen-9-yl)-1H-imidazo[4,5-f][1,10]phenanthroline (L^5)

Similar procedure was followed by taking 9-anthraldehyde.Yield: 35%; m.p.:179–181 °C; Anal. Calc. for $C_{27}H_{16}N_4$ (396.44 g/mol): Calc. (%):C, 81.80; H, 4.07; N, 14.13; Found (%): C, 81.67; H, 3.92; N, 14.01. ^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm: 13.587 (s, 1H, NH), 8.974–8.991 (complex, 2H, $H_{2',2''}$), 8.885 (d, 2H, $H_{4',4''}$), 8.521 (s, 1H, H_{A9}), 8.454 (d, 4H, $H_{A1,A4,A5,A8}$), 7.833 (t, 2H, $H_{3',3''}$), 7.440 (t, 4H, $H_{A2,A3,A6,A7}$), ^{13}C NMR ($CDCl_3$, 100 MHz): 154.15 ($C_{6',6''}$), 152.91 (C_2), 149.71 ($C_{2',2''}$), 134.33 (C_{A9}), 132.32 ($C_{4,5}$), 132.20 ($C_{A11,A12}$), 130.41 ($C_{A13,A14}$), 129.80 (C_{A10}), 128.17 ($C_{A4,A5}$), 126.45 ($C_{5',5''}$), 125.21 ($C_{A2,A3,A6,A7}$), 124.10 ($C_{A1,A8}$), 124.00 ($C_{4',4''}$), 121.41 ($C_{3,3''}$)

2-(Pyridin-2-yl)-1H-imidazo[4,5-f][1,10]phenanthroline [L^6]

Similar procedure was followed by taking pyridine-2-carbaldehyde. Yield: 23% m.p.:145–147 °C, anal. calc. for: $C_{18}H_{11}N_5$ (297.31 g/mol): calc. (%): C, 72.72; H, 3.73; N, 23.56, found (%): C, 72.64; H, 3.79; N, 23.49. ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm: 13.840 (NH, 1H) 9.023, (d, 2H, $H_{2',2''}$); 8.949, (d, 2H, $H_{4',4''}$); 8.814, (d, 1H, $H_{6'''}$); 8.139, (d, 1H, $H_{3'''}$); 7.984 (dt, 1H, $H_{4'''}$); 7.864–7.845, (complex, 3H, $H_{3',3'',5'''}$), ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 155.92, ($C_{6'''}$); 155.41, ($C_{2'''}$); 154.09, ($C_{6',6''}$); 150.12, (C_2); 149.15, ($C_{2',2}$.); 137.23, ($C_{3'''}$); 133.36, ($C_{5'''}$); 127.29, ($C_{4,5}$); 124.24, ($C_{5',5''}$); 123.12, ($C_{4',4}$.); 121.65, ($C_{4'''}$); 120.12($C_{3',3''}$); 120.12, (C_{ph6}).

2-(Pyridin-3-yl)-1H-imidazo[4,5-f][1,10]phenanthroline [L^7]

Similar procedure was followed by taking pyridine-3-carbaldehyde. Yield: 29% m.p.:141–143 °C, anal. calc. for: $C_{18}H_{11}N_5$ (297.31 g/mol): calc. (%): C, 72.72; H, 3.73; N, 23.56, found (%): C, 72.79; H, 3.67; N, 23.48. . ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm: 13.789, (NH, 1H), 9.219, (d, 2H, $H_{2',2''}$); 9.012, (s, 1H, $H_{2'''}$); 8.902–8.876, (complex, 3H, $H_{4',4'',6'''}$); 8.294, (d, 1H, $H_{4'''}$); 7.642, (t, 2H, $H_{Ph3',3''}$); 7.596, (d, 1H, $H_{5'''}$), ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 155.11, ($C_{2'''}$); 153.64, ($C_{6',6''}$); 149.65, (C_2); 148.94, ($C_{6'''}$); 135.44, ($C_{2',2''}$); 132.92, ($C_{4'''}$); 132.41, ($C_{3'''}$); 125.85, ($C_{4,5}$); 125.65, ($C_{5',5''}$)124.00, ($C_{5'''}$); 123.24, ($C_{4',4''}$); 121.21, ($C_{3',3''}$).

2-(Thiophen-2-yl)-1H-imidazo[4,5-f][1,10]phenanthroline [L^8]

Similar procedure was followed by taking thiophene-2-carbaldehyde. Yield: 23% m.p.:148–150 °C, anal. calc. for: $C_{17}H_{10}N_4S$ (302.35 g/mol): calc. (%): C, 67.53; H, 3.33; N, 18.53, found (%): C, 67.58; H, 3.29; N, 18.48. ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm: 13.521 (NH, 1H) 9.021, (d, 2H, $H_{2',2''}$); 8.847, (d, 2H, $H_{4',4''}$); 7.842, (d, 1H, H_{Th3}); 7.641(t, 2H, $H_{3',3''}$); 7.417 (d, 1H, H_{Th5}); 7.205, (t, 1H, H_{Th4}), ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 154.98, ($C_{6',6''}$); 148.92, (C_2); 145.84, (C_{Th2}); 141.79, ($C_{2',2''}$); 137.54, ($C_{4,5}$); 128.21, (C_{Th5}); 127.81, ($C_{5',5}$.); 127.49, (C_{Th4}); 123.89, ($C_{4',4''}$); 121.43, ($C_{3',3''}$); 120.52, (C_{Th3}).

2-Phenyl-1H-imidazo[4,5-f][1,10]phenanthroline [L^9]

Similar procedure was followed by taking benzaldehyde. Yield: 23% m.p.:135–137 °C, anal. calc. for: $C_{19}H_{12}N_4$ (296.33 g/mol): calc. (%): C, 77.01; H, 4.08; N, 18.91, found (%): C, 76.97; H, 4.01; N, 18.86. ^{1}H NMR ($CDCl_3$, 400 MHz) δ/ppm: 113.415 (NH, 1H) 9.075, (d, 2H, $H_{2',2''}$); 8.734, (d, 2H, $H_{4',4''}$); 7.942–7.911, (complex, 4H, $H_{3',3'',Ph2,6}$); 7.416–7.383, (m, 3H, $H_{Ph3,4,5}$), ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm: 155.37, ($C_{6',6''}$); 152.32, (C_2); 149.37, ($C_{2',2''}$); 134.72, (C_{ph1}); 132.45, ($C_{4,5}$); 131.12, (C_{ph4}); 129.59, ($C_{ph2,6}$); 127.21, ($C_{ph3,5}$); 126.78, ($C_{5',5''}$); 123.14, ($C_{4',4}$.); 121.32, ($C_{3',3''}$).

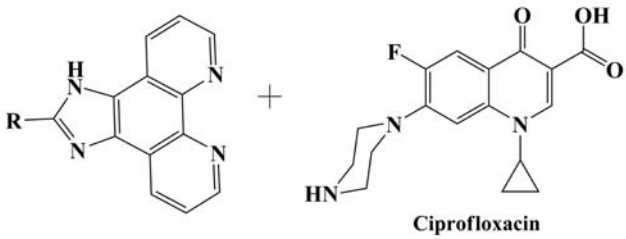
2. 3. Synthesis of Complexes

[Cu(cpf)(L^n)Cl]

Complexes were prepared by the reaction of ciprofloxacin (1.5 mmol), deprotonated with methanolic solution of CH_3ONa (1.5 mmol), $CuCl_2 \cdot 2H_2O$ (0.84 g, 1.5 mmol) in CH_3OH (15 mL) and different phenanthroline

Table 1: Experimental and physical parameters of the compounds

Complexes empirical formula	Formula weight (gm/mole)	Elemental analysis % found (required) C	H	N	M	m.p. °C	Yield %	μ_{eff} BM
$C_{36}H_{28}BrClCuFN_7O_3$(**1**)	804.55	53.68 (53.74)	3.44 (3.51)	12.24 (12.19)	7.81 (7.90)	245	63.5	1.81
$C_{36}H_{28}Cl_2CuFN_7O_3$ (**2**)	760.10	56.77 (56.89)	3.65 (3.71)	12.82 (12.90)	8.31 (8.36)	236	62.1	1.84
$C_{43}H_{35}ClCuFN_7O_4$ (**3**)	831.78	62.01 (62.09)	4.29 (4.24)	11.72 (11.79)	7.61 (7.64)	256	64.2	1.89
$C_{43}H_{35}ClCuFN_7O_4$ (**4**)	831.78	62.04 (62.09)	4.21 (4.24)	11.74 (11.79)	7.58 (7.64)	249	67.4	1.85
$C_{44}H_{33}ClCuFN_7O_3$ (**5**)	825.78	63.93 (64.00)	3.91 (4.03)	11.83 (11.87)	7.66 (7.70)	232	69.5	1.87
$C_{35}H_{28}ClCuFN_8O_3$ (**6**)	726.65	57.75 (57.85)	3.94 (3.88)	15.32 (15.42)	8.67 (8.75)	259	65.8	1.85
$C_{35}H_{28}ClCuFN_8O_3$ (**7**)	726.65	57.78 (57.85)	3.79 (3.88)	15.35 (15.42)	8.82 (8.75)	267	62.4	1.88
$C_{34}H_{27}ClCuFN_7O_3S$ (**8**)	731.69	55.73 (55.81)	3.64 (3.72)	13.47 (13.40)	8.57 (8.68)	252	65.2	1.83
$C_{36}H_{29}ClCuFN_7O_3$ (**9**)	725.66	59.68 (59.59)	3.96 (4.03)	13.44 (13.51)	8.69 (8.76)	239	68.9	1.84



R	Complex
3-Bromophenyl	1
3-Chlorophenyl	2
4-Benzyloxyphenyl	3
3-Benzyloxyphenyl	4
Anthracen-9-yl	5
Pyridin-2-yl	6
Pyridin-3-yl	7
Thiophine-2-yl	8
Phenyl	9

Scheme 1: General scheme of the structure of the complexes

derivatives (1.5 mmol) in CH_3OH (15 mL). The reaction mixture was refluxed for 2 h. A fine amorphous product of green color was obtained, which was washed with ether/hexane and dried in vacuum desiccator. The proposed reaction is shown in Scheme 1 and physical parameters of complexes shown in Table 1.

2. 4. Antibacterial Activity

All the complexes were assayed for their *in-vitro* antibacterial activity using the two fold broth dilution control having no any active ingredients, and DMSO as the solvent.[16] The antibacterial activity (MIC screening) of the compounds (ciprofloxacin, metal salt and complexes) was deliberated against two Gram$^{(+ve)}$ *Staphylococcus aureus, Bacillus subtilis* and three Gram$^{(-ve)}$ *Serratia marcescens, Escherichia coli* and *Pseudomonas aeruginosa*.

All equipment and culture media were sterilized. The incubation conditions comprise Luria Broth as media for culturing bacteria at 37 °C. This culture was used as a control to examine if the growth of the bacteria tested is normal. In a second set, 20 μL of the bacterial solution as well as the tested compound at the desired concentration were added. We monitored bacterial growth by measuring the turbidity of the culture after 18 h. If a certain concentration of a compound inhibited bacterial growth, half the concentration of the compound was tested. This procedure was carried on to a concentration that bacteria grow normally. The lowest concentration that inhibited bacterial growth was determined as the MIC value.

2. 5. DNA Interaction Activity

2. 5. 1. Viscosity Measurements

Viscosity measurements were carried out using an Ubbelohde viscometer maintained at a constant temperature of 27.0 ± 0.1 °C in a thermostatic bath. DNA samples with an approximate average length of 200 base pairs were prepared by sonication in order to minimize complexities arising from DNA flexibility.[17] Flow time was measured with a digital stopwatch. Each sample was measured three times and an average flow time was calculated. Data are presented as $(\eta/\eta_0)^{1/3}$ *versus* binding ratio ([Cu(II)]/[DNA]),[18] where η is the viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone.

2. 5. 2. Absorption Titration

Absorption titration experiments were performed by maintaining the metal complex concentration constant of 15 μM with varying the concentration of the CT DNA within 50–150 μM. While measuring the absorption spectra, equal quantity of CT DNA was added to all the complex solution and the reference solution to eliminate the absorbance of CT DNA itself. From the absorption data, the intrinsic binding constant K_b was determined from a plot of [DNA]/(ε_a–ε_f) *versus* [DNA] using Equation (1):

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

where [DNA] is the concentration of CT DNA in base pairs. The apparent absorption coefficients ε_a, ε_f and ε_b correspond to $A_{obsd.}$/[M], the extinction coefficient for the free metal(II) complex and extinction coefficient for the copper(II) complex in the fully bound form respectively.[19] K_b is given by the ratio of slope to the intercept.

2. 5. 3. Thermal Denaturation Experiments

Thermal DNA denaturation experiments were carried out with a Perkin–Elmer Lambda 850 spectrophotometer equipped with a Peltier temperature-control programmer (±0.1) °C. The temperature of the solution was increased from 30 to 90 °C at a rate of 5 °C min^{-1}, and the absorbance at 260 nm was continuously monitored for solutions of CT DNA (100 μM) in the absence and presence of the Cu(II) complexes (20 μM).

2. 6. DNA Cleavage Study

Cleavage of pUC19 DNA (50 μM) by complexes (200 μM) was measured by the conversion of supercoiled pUC19 DNA to open circular (OC) and linear (L). The % cleavage was calculated by equation below,

$$\% \text{ DNA cleavage} = \frac{[(\%SC\ DNA)control - (\%SC\ DNA)sample]}{(\%SC\ DNA)control} X\ 100$$

Gel electrophoresis of plasmid DNA (pUC19 DNA) was carried out in TAE buffer (0.04 M Tris–acetate, 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 3 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 ethidium bromide and was photographed on a UV illuminator. The percentage of each form of DNA was quantities using AlphaDigiDoc™ RT. Version V.4.1.0 PC–Image software.

2. 7. Determination of SOD-like Activity

SOD-like activity of all the complexes was determined by NBT/NADH/PMS system.[20] The superoxide radial produce by 79 μM NADH, 30 μM PMS, system containing 75 μM NBT, phosphate buffer (pH = 7.8), and 0.25 to 3.0 μM tested compound. The amount of reduced NBT was spectrophotometrically detected by monitoring the concentration of blue formazan form which absorbs at 560 nm. The reduction rate of NBT was measured in presence and absence of test compounds at various concentration of complex in the system. All measurements were carried out at room temperature. IC_{50} value of all the complexes was determined by plotting graph of percentage inhibition of NBT reduction against increase in concentration of the complex. Concentration of the complex which causes 50% inhibition of NBT reduction is reported as $IC_{50.}$

3. Result and Discussion

3. 1. Characterization of Complexes

All the complexes were analyzed using elemental analysis, magnetic measurements, reflectance, IR and FAB-mass spectrometry. The elemental analysis is in concurrence with proposed 1:1:1, metal:cpf:L^n formulation and theoretical expectation.

3. 2. IR Spectra

The determination of the coordinating atoms has been made by comparing IR spectra of the ciprofloxacin and metal complexes. Significant wave numbers are given in Table 2. The $\nu(C=O)_{carb}$ stretching vibration band appears at 1708 cm^{-1} for ciprofloxacin, where as for complexes, this band is replaced by $\nu(COO)_{as}$ and $\nu(COO)_s$ at 1563–1581 and 1342–1378 cm^{-1} respectively. The difference $\delta = \nu(COO)_{as} - \nu(COO)_s$ is useful for determining the coordination mode of ligands. The δ values are greater than 200 cm^{-1}, indicating monodentate coordination mode of carboxylato group[21–23] of the ligands. Strong absorption band at 1624 cm^{-1} in ciprofloxacin can be assigned for $\nu(C=O)_p$ vibration, while in metal complexes it appears at 1615–1628 cm^{-1}, which suggests that coordination occurs through carbonyl oxygen of pyridine ring. These data are further supported by ν(M-O) which appear at 505–513 cm^{-1} for complexes.

In the complexes the ν(C=N) band of phenanthroline appears at 1584 cm^{-1}. This band shift to higher frequency[24] at ~1626 cm^{-1} in complexes indicates bidentate N–N coordination of the ligand. N M bonding was supported by ν(M–N) band[25] at ~534–542 cm^{-1} for complexes.

3. 3. Reflectance Spectra and Magnetic Behaviour

The reflectance spectra of Cu(II) complexes exhibit one asymmetric broad band at around 15000 cm^{-1}, which suggest that compounds have a distorted square-pyramidal geometry.[26]

The magnetic moments of the copper(II) complexes lie in the 1.81–1.89 BM range. These values are typical of mononuclear copper(II) compounds with d^9 electronic configuration. The values are slightly higher than the expected spin-only values due to spin–orbit-coupling contribution.[27]

Table 2: IR spectra data

Compounds	ν(COO) Pyridone cm^{-1}	$\nu(COO)_{as}$ cm^{-1}	$\nu(COO)_s$ cm^{-1}	Δν cm^{-1}	ν(M-N) cm^{-1}	ν(M-O) cm^{-1}
Complex **1**	1621	1566	1364	202	534	512
Complex **2**	1615	1563	1342	221	541	509
Complex **3**	1628	1575	1378	197	536	505
Complex **4**	1612	1577	1374	203	542	503
Complex **5**	1615	1562	1363	199	539	513
Complex **6**	1627	1563	1366	197	532	519
Complex **7**	1620	1575	1379	196	537	516
Complex **8**	1629	1581	1377	204	546	519
Complex **9**	1623	1567	1372	195	543	513

3. 4. Mass Spectrometry

All the synthesized complexes show the molecular ion peak in FAB-MS spectra. In the spectra, we can clearly see the peak appears at 802, 804; 473, 475; and 429, 431 *m/z* assigned for the fragments of complex 1 having single Cl atom (Supply 2). Loss of chlorine atom give a fragment ion peak at *m/z* = 769, which also confirm that chlorine atom attached to metal ion with covalent bond. Several other peaks for fragments with *m/z* = 438, 393, 374 and 331 are found in spectra. Similar type of pattern is observed for rest of the synthesized complexes.

3. 5. Antibacterial Activity

Many coordination compounds have been studied for their antitumor,[28] antiviral[29] and antimalarial activity,[30] which has been related to the ability of metal ions to form stable complexes.[31] The results have led to an understanding of coordination sphere and electronic properties of the metal ions several workers have reported that heterocyclic rings containing sulfur, nitrogen, and/or oxygen are responsible for the biological activity of ligands and their metal complexes.[32]

The antibacterial activity of the cupric chloride, ciprofloxacin and its complexes were tested using double dilution method. An acceptable reason for this increase in antibacterial activity may be considered in the light of Overtone's concept[33] and chelation theory.[34] According to Overtone's concept of cell permeability, the lipid membrane that surrounds cell favors the passage of only lipid soluble materials, so that liposolubility is an important factor which controls bacteriostatic activity. On chelation, the polarity of the copper ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the copper ion with donor groups. Further, it increases the delocalization of π-electrons over whole chelate ring and enhances lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocks the metal binding sites in bacterial enzymes. These complexes also disturb the respiratory processes of the cell and thus block the synthesis of proteins which restricts further growth of the organism.

The results concerning *in vitro* antimicrobial activity (MIC) of ligands and their complexes are represented in Table 3. The antimicrobial activity of all complexes against five microorganisms is much higher than metal salt and much better than ciprofloxacin.

Table 3: MIC data of the compounds (μg/mL)

Compounds	*S. aureus*	*B. subtilis*	*S. marcescens*	*P. aeruginosa*	*E. coli*
$CuCl_2 \cdot 2H_2O$	15826	16512	16166	14101	19955
Ciprofloxacin	4.4	3.0	4.4	3.8	3.8
Complex **1**	1.1	1.1	1.1	1.0	1.4
Complex **2**	1.2	1.2	0.9	0.6	1.2
Complex **3**	1.6	1.7	1.4	1.7	1.2
Complex **4**	1.8	2.2	1.9	2.0	2.3
Complex **5**	2.3	2.4	2.3	2.5	2.2
Complex **6**	1.9	1.4	1.9	1.6	1.8
Complex **7**	2.1	1.2	2.1	1.8	1.9
Complex **8**	2.6	2.0	2.3	2.2	2.3
Complex **9**	2.9	2.6	2.9	2.6	2.6

3. 6. Viscosity Measurements

Optical photophysical probes provide necessary, but not sufficient clues to support a binding model. Hydrodynamic measurements (viscosity measurement) that are sensitive to length change are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data.[35–37] A classical intercalation model results in lengthening the DNA helix as base pairs are separated to accommodate the bound ligand, leading to the increase of DNA viscosity. In contrast, a semi-intercalation of ligand could bend (or kink) the DNA helix, reduce its effective length, and concomitantly, its viscosity. From Figure 1, the relative viscosities of the DNA solutions increased progressively with increase in the concentrations of all complexes. This is because of the fact that the molecules intercalate between the nucleobases of DNA base stacks and lengthen it, and thus raise the viscosity of the solution containing DNA. This proved that all complexes interact with DNA double strands via a classical interaction mode.[38] The order of viscosity of the compounds and ciprofloxacin is EtBr > 2 > 1 > 3 > 4 > 5 > ciprofloxacin. The binding ability of complexes is more compare to ciprofloxacin and less compare to classical intercalator ethidium bromide.[38]

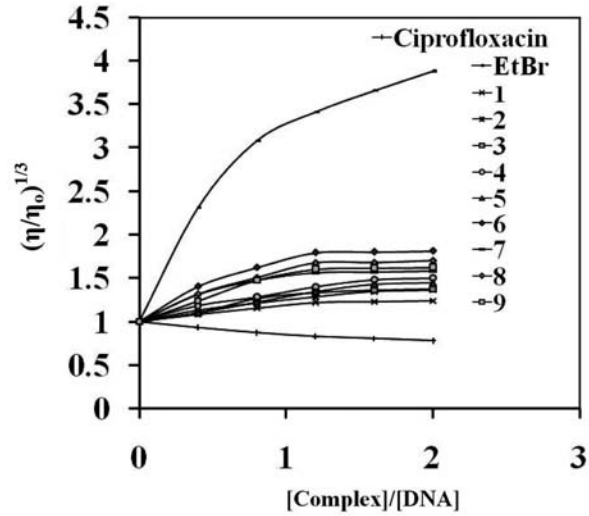


Figure 1: Effect on relative viscosity of DNA under the influence of increasing amount of complexes at 27 ± 0.1 °C in in 5 mM Tris–HCl buffer (pH 7.2) as a medium.

3. 7. Absorption Titration

Complex binding with CT-DNA through intercalation usually result in hypochromism and bathochromism, due to intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA.[39,40] The binding of Cu(II) complexes to duplex DNA led to decrease in the absorption intensities with a small amount of red shifts in the UV–Vis absorption spectra. After intercalation, the π^* orbital of the intercalated ligand can couple with the π orbital of nucleobases of DNA base stacks, thus decreasing the $\pi - \pi^*$ transition energy and resulting in the bathochromism.[41,42]

On the other hand, the coupling π orbital is partially filled by electrons, thus decreasing the transition probabilities resulting in the hypochromism. The binding constant (K_b) of the complexes to DNA were determined by monitoring the changes of absorbance at 281–284 nm with increasing concentration of DNA (Figure 2). The appreciable decrease in absorption intensity and significant red shift of the $\pi - \pi^*$ band of complexes is similar to that observed for its interaction with DNA in DMSO solution. The binding constant (K_b) of complexes (Table 4) are in the range of 1.12×10^4 – 3.05×10^4. Comparing the intrinsic binding constant of complexes with those of DNA–intercalative $[Ru(dmb)_2(ipbp)]^{2+}$ ($1.18 \times 10^4\ M^{-1}$) complex[43] [where, dmb = 4,4'-dimethyl-2,2'-bipyridine and ipbp = 3-(1H-imidazo[4,5-f][1,10]phenanthroline-2-yl)-4H-1-benzopyran-2-one]. The K_b value of complexes, is greater than Co(II) complexes of terpyridines reported by Indumathy et al.[44] and Cu(II) complexes of type $[Cu(phen)_2Cl_2]$[45] while comparable to ruthenium complexes reported by Tan et al.[46]. So we can deduce that all the complex bind moderately to DNA by intercalation. These spectral characteristics obviously suggest that complexes interact with chromophore and the nucleobases of DNA base stacks.

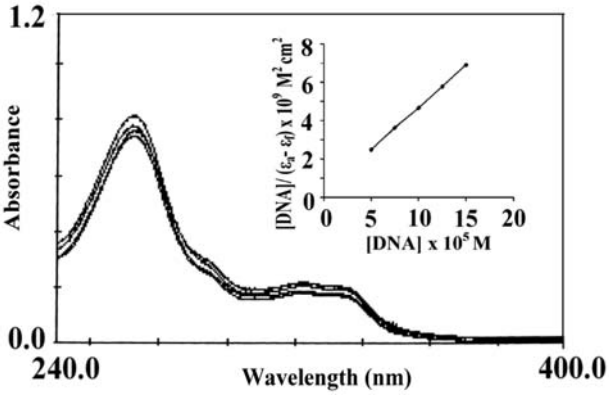


Figure 2: Electronic absorption titration curve of [Cu(cpf)(L^1)Cl] in absence and in presence of increasing amount of DNA; 50–150 μM in in 5 mM Tris–HCl buffer (pH 7.2), [Complex] = 15 μM, [DNA]= 50–150 μM with incubation period of 30 min. at 37 °C, Inset: Plot of [DNA]/(ε_a–ε_f) *versus* [DNA].

Table 4: Binding constant and IC_{50} values of copper(II) complexes

Compounds	K_b (M^{-1})	IC_{50} (μM)	T_m
Complex **1**	2.08×10^4	0.70	79.4
Complex **2**	3.05×10^4	0.60	80.1
Complex **3**	2.05×10^4	0.90	78.9
Complex **4**	1.59×10^4	1.20	78.5
Complex **5**	1.12×10^4	1.45	78.3
Complex **6**	1.65×10^4	0.95	78.9
Complex **7**	1.57×10^4	1.20	78.6
Complex **8**	1.31×10^4	1.50	78.2
Complex **9**	1.12×10^4	1.75	78.5

3. 8. Thermal Denaturation Experiment

The melting of DNA is an important parameter to study the interaction of transition metal complexes with nucleic acids. Thermal behaviours of DNA in the presence of complexes can give an insight into their conformation changes when temperature is raised, and offer information about the interaction strength of complexes with DNA. The melting temperature T_m, at which 50% of the DNA has become single strand, can be determined from the thermal denaturation curves of DNA by monitoring the absorption changes at 260 nm. According to the literature,[47–49] the intercalation of metallointercalators generally results in a considerable increase in melting temperature (T_m). In the absence of the complex, a DNA–melting experiment showed that T_m of CT DNA (100 μM) is 74.2 ± 0.1 °C under experimental conditions (Supply. 3). The observed melting temperatures of DNA in presence of complexes (T_m) are shown in Table 4. The increase amount of T_m is comparable to that observed for classical intercalators.[47–49]

3. 9. Gel Electrophoresis

Isolation of plasmid DNA from pure culture of *E. coli* was carried out by conventional method.[50] The basic principle employed is "alkali-lysis", in which at the alkaline pH, both the genomic and plasmid DNA are denatured. On reduction of the pH the plasmid DNA molecule being small in size, quickly reanneals itself while the large genomic DNA is not. The denatured genomic DNA is then sedimented while the plasmid DNA remains in solution. This is then precipitated.

Cleavage of plasmid pUC19 DNA by synthesized complexes was monitored by agarose gel electrophoresis technique. When plasmid DNA was subjected to electrophoresis after interaction, upon illumination of gel (Figure 3) the fastest migration was observed for super coiled (SC) Form I, where as the slowest moving was open circular (OC) Form II and the intermediate moving is the linear (L) Form III generated on cleavage of open circular. The data of plasmid cleavage are presented in Table 5. All the complexes show higher DNA cleavage ability compare to the drug and metal salt.

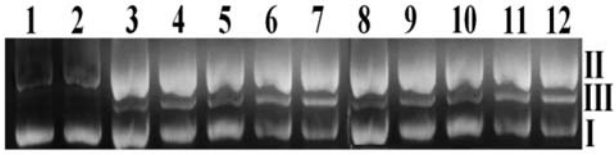


Figure 3: Photogenic view of interaction of pUC19 DNA (450 μg/mL) with series of copper(II) complexes (200 μM) using 1% agarose gel containing 0.5 μg/mL ethidium bromide. All reactions were incubated in TE buffer (pH 8) in a final volume of 15 μL, for 3 h. at 37 °C. : Lane 1, DNA control; Lane 2, $CuCl_2 \cdot 2H_2O$; Lane 3, ciprofloxacin; Lane 4, [Cu(cpf)(L^1)Cl]; Lane 5, [Cu(cpf)(L^2)Cl]; Lane 6, [Cu(cpf)(L^3)Cl]; Lane 7, [Cu(cpf)(L^4)Cl]; Lane 8[Cu (cpf)(L^5)Cl], [Cu(cpf)(L^6)Cl], [Cu(cpf)(L^7)Cl], [Cu(cpf)(L^8)Cl], [Cu(cpf)(L^9)Cl].

Table 5: Gel electrophoresis data.

Compounds	% SC	% OC	% L	% Cleavage
DNA Control	75	25	–	
DNA + Metal salt	72	28	–	4.00
DNA + Ciprofloxacin	67	33	–	10.66
DNA + **1**	18	52	30	76.00
DNA + **2**	16	52	32	78.66
DNA + **3**	20	51	29	73.33
DNA + **4**	26	54	20	65.33
DNA + **5**	30	55	15	60.00
DNA + **6**	22	57	20	74.41
DNA + **7**	26	55	17	69.76
DNA + **8**	28	53	19	67.44
DNA + **9**	30	42	28	65.11

3. 10. SOD Mimic Activity

Superoxide is one of the main reactive oxygen species (ROS) in the cell and as such, SOD serves a key antioxidant role. SOD outcompete damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is spin forbidden in biological systems; this means, its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO). The superoxide anion radical ($O_2^{\bullet -}$) spontaneously dismutes to O_2 and hydrogen peroxide (H_2O_2) quite rapidly (~10^5 $M^{-1}s^{-1}$ at p-H 7). SOD is biologically necessary because superoxide reacts even faster with certain targets such as the NO radical, which makes peroxynitrite.

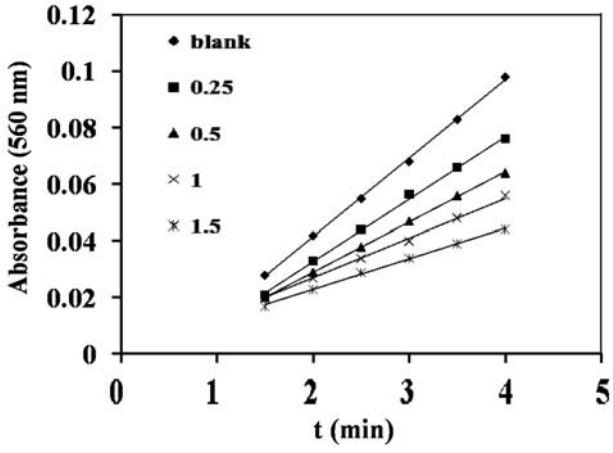


Figure 4: plot of absorbance values(Abs_{560}) against time (t).

The system used as a basis of superoxide radical generator in order to check SOD like activity of the synthesized complexes was NBT/NADH/PMS system. Absorbance at a function of time was plotted to have a straight line obeying equation Y = mX + C (Figure 4). Figure 5 shows percentage inhibition of reduction of nitro blue tetrazolium (NBT) plotted against concentration of the complex 1. Compounds exhibit SOD–like activity at biological pH with their IC_{50} values ranging from 0.60 to 1.75 μM. The superoxide scavenging data are presented in Table 4. The higher IC_{50} can only be accredited to the vacant coordination site facilitating the binding of superoxide anion, electrons of aromatic ligands that stabilize $Cu–O_2^{\cdot -}$ interaction and not only to the partial dissociation of complex in solution.

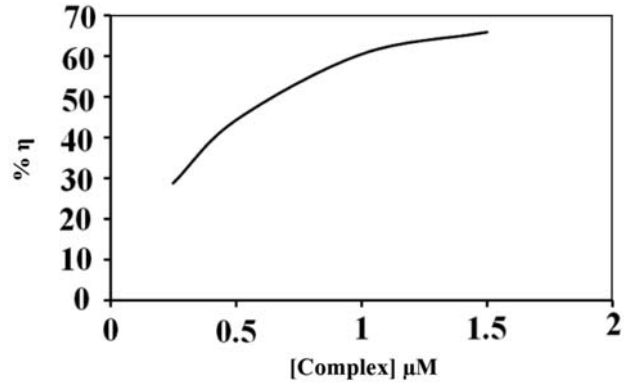


Figure 5: Plot of percentage of inhibiting NBT reduction with an increase in the concentration of complex 1.

4. Conclusion

The second generation fluoroquinolone drug, ciprofloxacin, based neutral mixed ligand complexes of copper(II) with various phenanthroline derivatives have been synthesized. The spectroscopic evidences suggest square pyramidal geometry around Cu(II) ions. All the complexes were screened for diverse biological activities to check their possible applications as antibacterial or SOD mimic agents. Antibacterial activity shows better MIC value of all complexes than ciprofloxacin against different microorganisms. Complexes show moderate activity for DNA interaction assay. Also complexes show good SOD mimic activity for nonenzymatic NADH/PMS/NBT assay. Although complexes does not have very much structural differences, but based on the trends observed in the biological activities, we can say that complexes

with electron withdrawing groups and with larger ring system show good biological activities by stabilizing the charge of central metal ion over the extended ring systems.

6. References

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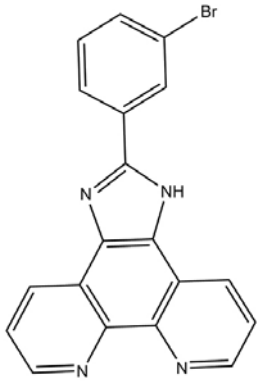
Povzetek

Raziskavam interakcij majhnih molekul (ligandov) z DNK se v zadnjem času posveča veliko pozornosti. V tem delu smo sintetizirali smo ligande bakrovih (II) kompleksov tipa [Cu(cpf)(L^n)Cl] [cpf = ciprofloksacin, L^n = derivati fenantrolina]. Njihovo sestavo smo preverili z elementno in masno analizo, meritvami reflektance ter IR spektroskopijo. Naravo vezanja bakrovih (II) kompleksov na DNK smo študirali z merjenjem viskoznosti ter merjenjem titracijskih in talilnih krivulj nastalih kompleksov s pomočjo UV-Vis absorpcije. Raziskave cepitve DNK so pokazale, da imajo sintetizirani bakrovi (II) kompleksi večjo sposobnost cepitve DNK kot soli kovin in običajna zdravila. Za proučevane komplekse smo izvedli tudi raziskave mimike superoksid dismutaze (SOD) ter določili vrednosti IC_{50} v območju med 0,95 in 1,75 μM. Antibakterijska aktivnost smo testirali na izbranih grampozitivnih in gramnegativnih mikroorganizmih.

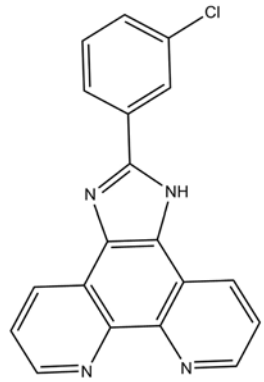
Supplementary material

Supplementary 1. Structure and name of ligands



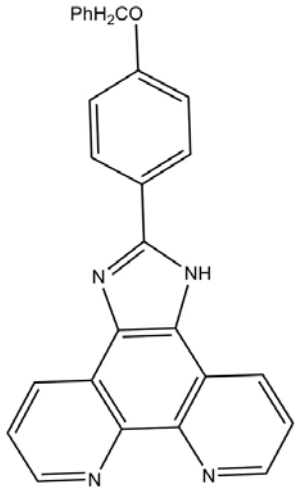
2-(3-bromophenyl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline

Ligand 1 (L^1)



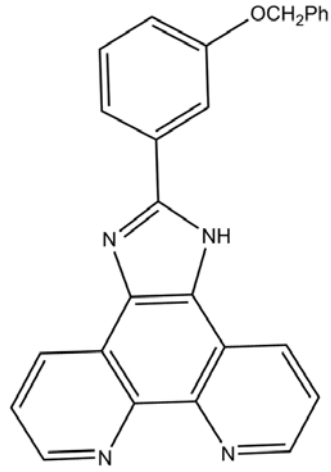
2-(3-chlorophenyl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline

Ligand 2 (L^2)



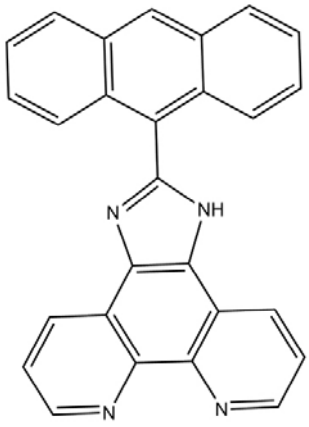
2-(4-(benzyloxy)phenyl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline

Ligand 3 (L^3)



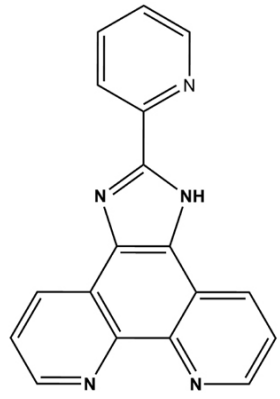
2-(3-(benzyloxy)phenyl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline

Ligand 4 (L^4)



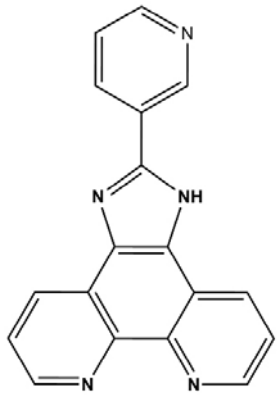
2-(anthracen-9-yl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline

Ligand 5 (L^5)



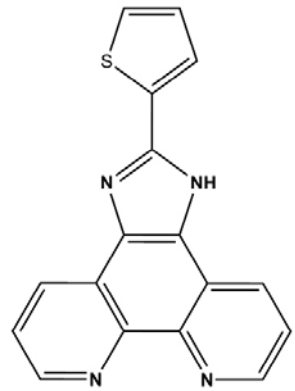
2-(pyridin-2-yl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline

Ligand 6 (L^6)



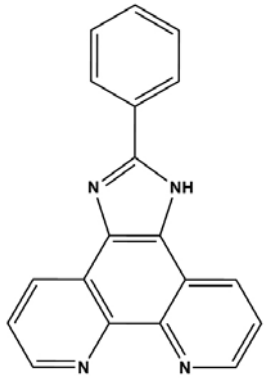
2-(pyridin-3-yl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline

Ligand 7 (L^7)



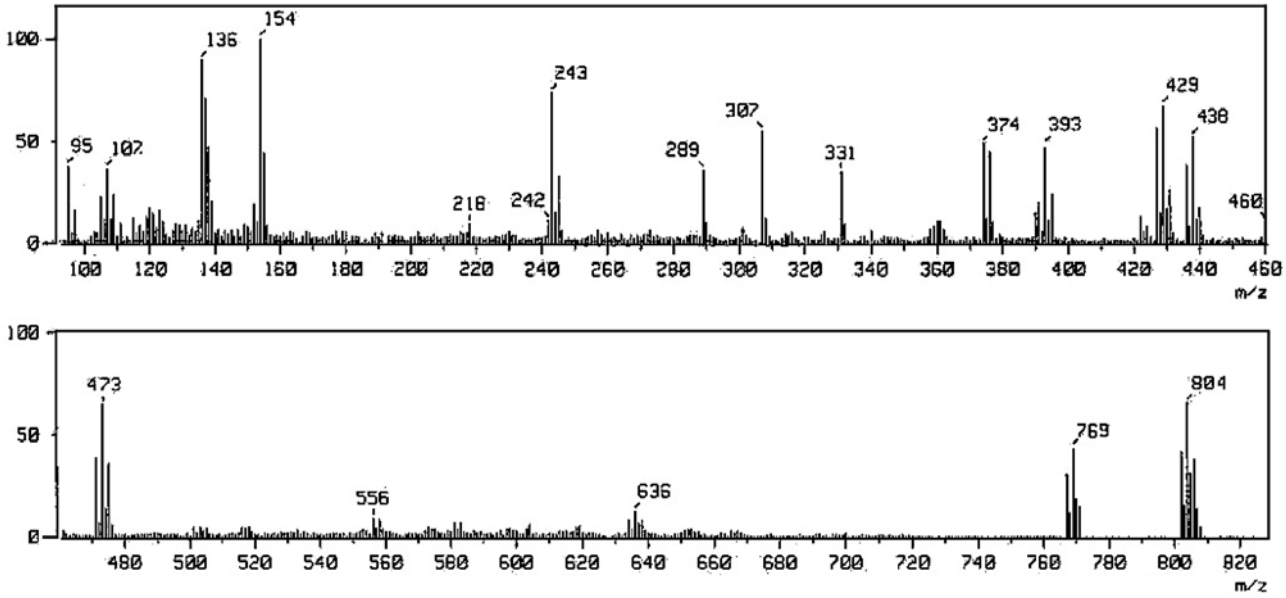
2-(thiophen-2-yl)-1*H*-imidazo[4,5-*f*][1,10]phenanthroline

Ligand 8 (L^8)

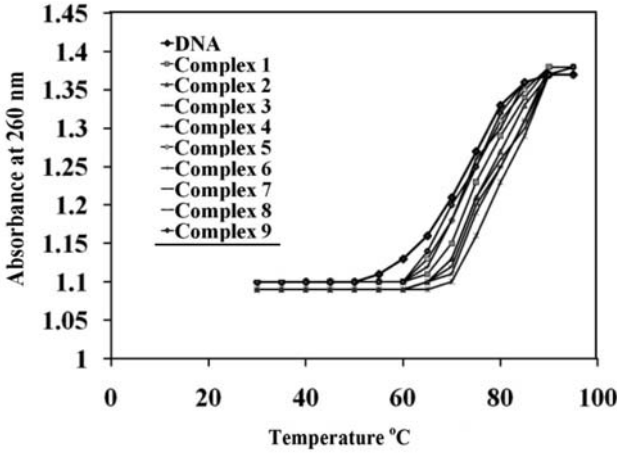


2-phenyl-1*H*-imidazo[4,5-*f*][1,10]phenanthroline

Ligand 9 (L^9)



Supplementary 2. FAB-mass spectrum of complex 1, that is [Cu(cpf)(L^1)Cl], obtained using *m*–nitro benzyl alcohol.



Supplementary 3. Melting curves of CT DNA in the absence and presence of complexes 1–9.